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DISENTANGLING THE BODY MASS INDEX, METABOLIC HEALTH, AND AGING CONNECTION: *WEIGHTY MATTERS*

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Disentangling the Body Mass Index, Metabolic Health and Aging Connection: Weighty Matters

Thesis for Doctoral Degree (Ph.D.)

By

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To Bǎbá. Although most people say I've got your looks, I think I've got your brains too. Without your emphasis on diligence, creativity, and problem-solving while I was growing up, I wouldn't have had the tenacity to complete a PhD.

To my two Sisters: Because you are much older, I had the good fortune to be taught not only in school but also by you at home while growing up.

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To all the girls and boys who have missed the chance to continue their education, not because of the lack of potential but because of lack of opportunity — may opportunities come. Never say never.

Preface

When I first thought about pursuing a PhD, I never imagined I'd end up in the obesity and aging research field. What's serendipitous, though, is that the first popular science article I ever wrote, way back when I was a young respiratory therapist, was something related to obesity. I also remember sitting in my respiratory therapy classes in college, where most of my classmates were excited about specializing in pediatrics or neonatal, working with children and newborns — it seemed so cool then. But, secretly, the 20-year-old me thought, "I want to work with older people."

None of this would have been possible without Ida and Anna, who took a chance on me during a Zoom interview in the early days of the COVID-19 pandemic. Despite all the uncertainty at that time, they saw potential in me before I saw it in myself, and for that, I am deeply grateful. They opened the door to this incredible journey and guided me every step of the way, making sure I saw it through to the end.

Throughout this journey, my co-supervisors — Alex, Debbie, and Juulia — played key roles. Each brought their own expertise and unique strengths, and their support made all the difference. I couldn't have asked for a better team to help guide me through the twists and turns of this research.

This work wouldn't exist without the dedication of countless scientists before me. Their discoveries paved the way, and I am incredibly grateful for the opportunity and privilege to learn from the foundation they built. I also want to acknowledge all the researchers who established and maintained the cohorts, those who meticulously collected the data, and the participants who generously gave their time — without them, this thesis wouldn't even be imaginable.

I have to mention Robert Lustig's YouTube video, "Sugar: The Bitter Truth." My boyfriend, Michel, encouraged me to watch it, and that simple suggestion sparked a curiosity that eventually grew into a full-blown obsession with obesity and metabolic health — ultimately leading to this thesis. The truth is, bitter or sweet, I used to think, "I will never want to study this again," after struggling to memorize all those endocrine pathways back in college (well, lungs are more my thing, you see). I never would have guessed I needed to study these pathways again. Fortunately, there are now many videos online that make complex topics

accessible. Imagine if this were the eighties or nineties; I doubt I would have even made it to a PhD program.

In this thesis, I explore how body mass index and metabolic health relate to biological aging and mortality across four studies. Chapter 1 provides the background on BMI, metabolic health, and biological aging. Chapter 2 lays out the research aims. Chapters 3 and 4 get into the technical details, with Chapter 3 covering the materials and methods and Chapter 4 diving into each study's methods, results, and challenges. Finally, Chapters 5 through 7 wrap everything up with discussion, conclusions, and personal reflections.

Perhaps this thesis adds a small contribution to the body of knowledge on body mass index, metabolic health, and aging, but what's more meaningful to me is the journey and the collaborative spirit of everyone who supported me along the way. This work is truly the result of many hands and minds coming together, and I am deeply grateful for all the support I've received, and the foundation laid by those who came before me.

Abstract

As global populations age and the prevalence of obesity and metabolic disorders rises, understanding the complex relationships between body mass index (BMI), metabolic health, and aging becomes increasingly critical. This thesis sought to unravel these connections, focusing on how BMI and metabolic health are associated with biological aging and all-cause mortality while considering the nonlinear effects of BMI and age differences. By employing measures of biological aging — encompassing functional (functional aging Index, FAI), physiological (frailty index – FI), and cellular (epigenetic age acceleration – EAA) levels — we aimed to provide a comprehensive examination of the BMI, metabolic health, and biological aging connections.

Study I examined the independent and joint associations of midlife and late-life BMI and metabolic health status (MHS) assessments with risk of all-cause mortality. Data from 6,252 Swedish twins in midlife (65 years and below) and 6,215 in late life (over 65 years) were analyzed using Cox proportional hazards models. In the joint models, being metabolically unhealthy (MU) was consistently associated with increased mortality risk robust to BMI adjustments, while the mortality risk associated with BMI categories attenuated. In the interaction models, MU with obesity in midlife and across all BMI categories in late life was associated with higher mortality risk than metabolically healthy normal weight (MHN). Conversely, metabolically healthy with overweight (MHOw) or obesity in midlife and late life was not associated with higher mortality risks. In fact, late-life MHOw was associated with a lower mortality risk compared to MHN. These findings suggest that MHS plays a more significant role than BMI in predicting mortality risk.

Study II investigated how BMI and MHS jointly associate with biological aging, measured by FAI and FI, and whether these associations varied by chronological age. A cross-sectional analysis of 1,825 Swedish twins using mixed-effects linear models revealed a U-shaped association between BMI and FAI, where low and high BMI were associated with higher biological aging. MU was also associated with higher FAI. Significant three-way interactions between BMI, MHS, and chronological age on FI prompted the stratification of the analysis by age: below 65, 65 to 85, and over 85 years. In these groups, low BMI, high BMI and MU were consistently associated with greater FI, with significant modifications by MU and chronological age in the 65 to 85 and over 85 groups, respectively. This study

highlights a complex interplay between BMI, MHS, and chronological age. Low BMI, high BMI, and MU were associated with higher biological aging, indicating their potential contribution to age acceleration.

Study III explored if biological aging, measured by EAA, mediates the BMI-mortality relationship. Using data from 3,840 participants in the U.S. Health and Retirement Study, a nonlinear association was found: both low and high BMI were associated with increased EAA and shorter life expectancy. Mediation analysis showed that high BMI's association with shorter life expectancy was strongly mediated by EAA, supporting the hypothesis that obesity accelerates biological aging. In contrast, the association of low BMI with shorter life expectancy was mainly driven by direct effects rather than mediation through biological aging.

Study IV analyzed the bidirectional relationship between change in BMI and biological aging, measured by FAI and FI, in 1,902–1,976 Swedish twins aged 60 to 91.9, using dual change score models. The age trajectory of BMI followed an almost linear, declining pattern, whereas FAI and FI exhibited exponentially increasing trends. The study found a unidirectional relationship where higher FAI predicted a steeper BMI decline. In contrast, the BMI-FI relationship was bidirectional — higher BMI predicted increased FI and higher FI contributed to a steeper BMI decline.

These findings underscore the complex nature of the relationships between BMI, metabolic health, and aging, revealing the distinct influences of high BMI, low BMI, and metabolic health on biological aging and life expectancy. Together, these results emphasized the importance of integrating BMI, metabolic health, and biological aging into the assessment of late-life health, offering new insights into how these factors may converge to potentially shape the aging process and survival.

Keywords: aging, all-cause mortality, biological aging, body mass index, frailty, epigenetic age, metabolic health, obesity, mediation analysis, dual change score models

List of scientific papers

I. **Ler P**, Li X, Hassing LB, Reynolds CA, Finkel D, Karlsson IK, Dahl Aslan AK. Independent and joint effects of body mass index and metabolic health in mid- and late-life on all-cause mortality: a cohort study from the Swedish Twin Registry with a mean follow-up of 13 years. BMC Public Health. 2022;22(1):718

II. **Ler P**, Ploner A, Finkel D, Reynolds CA, Zhan Y, Jylhävä J, Dahl Aslan AK, Karlsson IK. Interplay of body mass index and metabolic syndrome: association with physiological age from midlife to late-life. Geroscience. 2024;46(2):2605 – 2617

III. **Ler P**, Jylhävä J, Finkel D, Aslan Dahl AK, Ploner A, Karlsson IK. Does biological aging mediate the association between body mass index and survival among older adults? (Manuscript)

IV. **Ler P**, Mak JKL, Reynolds CA, Ploner A, Pedersen NL, Jylhävä J, Aslan Dahl AK, Finkel D, Karlsson IK. Longitudinal study of body mass index and biological aging: investigating the temporal dynamics. (Submitted)

Related papers not included in the thesis

Ler P, Ojalehto E, Zhan Y, Finkel D, Dahl Aslan AK, Karlsson IK. Conversions between metabolically unhealthy and healthy obesity from midlife to late-life. International Journal of Obesity (London). 2024; 48(3):433 – 436

Contents

List of abbreviations

ACME	Average causal mediation effects
ADE	Average direct effects
AIC	Akaike information criteria
BA	Biological age measures
BMI	Body mass index
CI	Confidence interval
CVD	Cardiovascular disease
DALY	Disability-adjusted life-years
DCSM	Dual change score model
DunedinPACE	Rate of biological aging in year per chronological year
EAA	Epigenetic age acceleration
FAI	Functional aging index
FI	Frailty index
GENDER	Aging in Women and Men: A Longitudinal Study of Gender Differences in Health Behaviour and Health among Elderly
GrimAgeAcc	Acceleration of GrimAge
HannumAgeAcc	Acceleration of Hannum epigenetic clock
HbA1c	Hemoglobin A1c or glycated hemoglobin levels
HDL-C	High-density lipid-cholesterol
HorvathAgeAcc-I	Acceleration Horvath's first epigenetic clock
HorvathAgeAcc-II	Acceleration of Horvath's epigenetic clock enhanced for skin, fibroblasts, and blood
HRS	Health and Retirement Study
HRs	Hazard ratios
IPT	In-person testing
LRT	Likelihood ratio tests

MetS	Metabolic syndrome
MHN	Metabolically healthy normal weight
MHO	Metabolically healthy obesity
MHOw	Metabolically healthy overweight
MHS	Metabolic health status
MU	Metabolically unhealthy
MUN	Metabolically unhealthy normal weight
MUO	Metabolically unhealthy obesity
MUOw	Metabolically unhealthy overweight
NCDs	Non-communicable diseases
NCEP ATP-III	National Cholesterol Education Program Adult Treatment Panel-III
OCTO-Twin	Origins of Variances in the Oldest-Old: Octogenarian Twins
PEF	Peak expiratory flow
PhenoAgeAcc	Acceleration of PhenoAge
RCS	Restricted cubic splines
SAT	Subcutaneous adipose tissue
SATSA	Swedish Adoption/Twin Study of Aging
SD	Standard deviation
SEV	Summary Exposure Values
STR	Swedish Twin Registry
T2DM	Type II diabetes mellitus
VAT	Visceral adipose tissue
WC	Waist circumference
WHR	Waist-hip ratio

1 Introduction

The world is undergoing profound epidemiological and demographic transitions, marked by the alarming rise in the prevalence of obesity and metabolic disorders alongside an aging population. Between 1990 and 2022, the global prevalence of adult obesity surged from 7% to 16%,[1] while the exposure prevalence to metabolic risks, which included high body mass index (BMI), hypertension, hyperglycemia, and dyslipidemia, increased from an average of 13% to 21%.[2] Concurrently, the proportion of the global population aged 65 nearly doubled, rising from 6% to 10% during the same period.[3, 4] Given that obesity and metabolic dysfunction are closely related to age-related diseases,[2, 5, 6] these escalating trends present critical global health challenges, compelling further investigation into the intricate relationships between obesity, metabolic health, and aging.

Although global healthy life expectancy has improved, the overall burden of disease has increased, with non-communicable diseases as a critical contributor and type 2 diabetes (T2DM) among the top climbers in 2021.[7] This growing burden is largely driven by population growth and aging.[7] This disparity — where longer life expectancy does not necessarily translate to better health — highlights the urgent need to understand how obesity and metabolic health influence healthy aging. Understanding these interrelationships is crucial for managing the growing burden of chronic diseases and improving the health of the aging population.

However, examining the link between obesity and late-life health is complicated by the 'obesity paradox' — the concept that high BMI in late life may be associated with better health outcomes, contrary to the well-established negative health impact of high BMI in early to mid-adulthood. While solid evidence shows that high BMI in younger and middle-aged adults is a risk factor for mortality, [8-10] its effects in older age remain less clear.[11-14] In fact, some studies suggest that high BMI may be associated with better survival in older adults.[11-14] This paradox highlights the need to explore why high BMI in late life may have different health implications and how it influences health outcomes among older adults.

Adding to the complexity of the connection between BMI, metabolic health, and aging is the concept of "metabolic healthy obesity" (MHO). - Despite the common association of high BMI with metabolic diseases, approximately 35% of individuals with obesity do not exhibit signs of metabolic dysfunction.[15] The state of obesity with preserved metabolic health, known as MHO,[15–19] raises questions about whether it represents a less detrimental form of obesity.[20–22] The limited research

on MHO in older populations adds to the uncertainty about its impact on health outcomes and its role within the obesity paradox and aging. Furthermore, metabolic diseases affect individuals across the BMI spectrum, making it essential to consider metabolic health alongside BMI in studies investigating their connections with aging.

Aging is traditionally and most commonly assessed as chronological age, which assumes a uniform rate of aging across all individuals. This approach, however, overlooks the heterogeneity in health and aging processes.[23] To address this limitation, researchers increasingly turn to biological age measures, which evaluate functional, physiological, and cellular markers to capture variations and nuances during aging. Incorporating biological aging metrics helps to disentangle the complex relationships between BMI, metabolic health, and biological aging by providing a more comprehensive view of how these factors interact and influence health outcomes throughout the aging process.

Amidst the triple burden of global health — an obesity epidemic, the rising prevalence of metabolic diseases, and the aging population — this thesis seeks to disentangle the complex interplay between BMI, metabolic health, and aging. Through a literature review and four studies, each meticulously examining these interconnections and using cohort data from Sweden and the United States of America (U.S.), the research seeks to shed light on the roles these factors play in the aging process. The ultimate goal of epidemiological studies is often to inform the development of effective strategies for improving health outcomes. Realistically, this thesis focuses on deepening our understanding of these closely intertwined factors and highlighting key questions and considerations for managing BMI and metabolic health in older populations. Ultimately, this work represents the tip of the iceberg of knowledge and information, the beginning of a broader inquiry, marking just the start of what needs to be an ongoing exploration.

1.1 The Weighty Matters

1.1.1 Epidemiology of Overweight and Obesity

The prevalence of overweight and obesity has surged substantially over the past decades worldwide. Between 1975 and 2016, the number of adults classified as overweight grew by roughly 1.3 billion, while those classified as obesity rose by approximately 571 million.[24] The age-standardized prevalence of overweight and obesity in 1975 stood at 26.5% and 7%, respectively.[25] By 2015, these figures had soared to 39% and 12.5%, reflecting 50% and 80% increases.[25] While there are regional differences, the prevalence of overweight and obesity increased in every country in 2016 and 2019.[24, 26] Figure 1 maps the prevalence of adult overweight or obesity in 2016 and 1975.

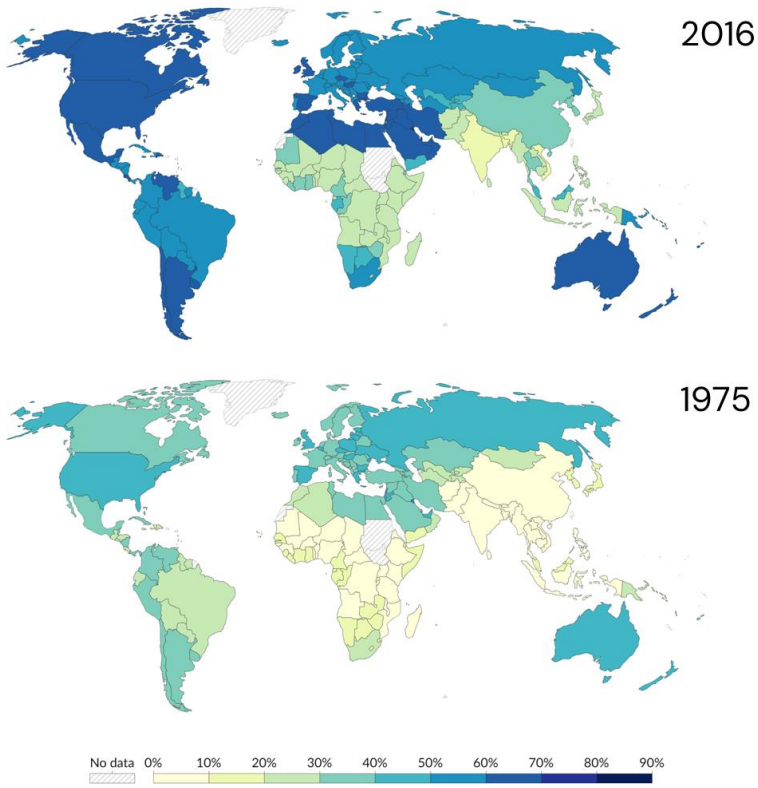

Figure 1: Mapping the Prevalence of Adult Overweight or Obesity in 2016 versus 1975[27]

The proportion of adults 18 years and above with BMI is 25kg/m^2 or higher in percentage. Image modified from https://ourworldindata.org/grapher/share-of-adults-who-are-overweight[27]

The impact of high BMI on the global disease burden has been increasing over time. From 1990 to 2015, the global mortality rate attributable to overweight and obesity rose by 28%.[28] Recent research has shifted its focus from reporting prevalence rates to utilizing more nuanced metrics like Summary Exposure Values (SEV) to understand disease burdens.[2] SEV measures the age-standardized, risk-weighted prevalence of exposure on a scale from 0 to 100, with 100 indicating maximum exposure for the entire population. From 1990 to 2021, the SEV for high BMI, defined as BMI greater than 23.0 kg/m^2, rose from 12.6 to 21.5 (on a scale of 100), reflecting an increase in prevalence and overall burden.[2] During the same period, the global age-standardized rates of health loss from disability and premature mortality, measured as disability-adjusted life-years (DALY), attributable to high BMI increased from 2.5% to 4.5%, underscoring the growing health impact of high BMI.[2]

Overweight and obesity rates typically increase throughout adulthood, peaking between the ages of 50 and 65.[25] In 2021, the global DALY rate attributable to high BMI was estimated to be 3.2% among individuals aged 15 to 49 years.[2] The global DALY rates nearly doubled to 7.6% for those aged 50 to 69.[2] Among individuals aged 70 years and above, DALY rates from high BMI slightly decreased to 6.2%. Although this is lower than the rate observed in the 50 to 69 age group, it remains higher than the age-standardized rates and the rates for those aged 15 to 49.[2] Therefore, the global disease burden from high BMI appears to increase with age.

1.1.2 Population Health, Metabolically Speaking

High BMI is one of many metabolic risk factors. Other metabolic risk factors include hypertension, hyperglycemia, and dyslipidemia. According to two consecutive Global Burden of Disease Studies, one published in 2020 and the other in 2024, hyperglycemia based on high fasting plasma glucose levels and high BMI has demonstrated the largest increments in exposure prevalence based on SEVs.[2, 26] The exposure prevalence of hyperglycemia increased from 10.6 in 1990 to 16.2 (on a scale of 100) in 2021.[2] Additionally, exposure prevalence of hypertension, defined as high systolic blood pressure, increased from 33 in 1990 to 35.6 (on a scale of 100) in 2021.[2] While dyslipidemia, based on low levels of low-density lipid cholesterol levels, showed a slight decline in exposure prevalence of 0.1%, the global exposure prevalence in 2021 remains high at 45.3 (on a scale of 100).[2]

In 2000 and 2021, hypertension, hyperglycemia, and dyslipidemia consistently ranked among the top nine leading risk factors for disability and mortality out of 88 environmental, occupational, and behavioral risk factors.[2] The proportion of total disease burden, quantified by DALYs and attributed to hypertension, climbed from 6.3% in 2000 to 7.8% in 2021, establishing it as the second largest contributor to the global disease burden.[2] Similarly, the impact of hyperglycemia and dyslipidemia on global health also rose during this period, with their contributions growing from 3.1% to 5.4% and from 2.6% to 3.0%, respectively.[2] By 2021, hyperglycemia had become the fifth leading risk factor, and dyslipidemia the seventh, for disability and mortality worldwide.[2]

By analyzing the global burden of diseases in relation to changes in risk exposure, population growth, and aging population, evidence suggests that the significant contribution of metabolic risk factors — such as high BMI, hypertension, and hyperglycemia — to morbidity and mortality is primarily driven by global population aging.[2] Notably, the impact of hypertension, hyperglycemia, and dyslipidemia increases with advanced age. Among individuals aged 15 to 49, 50 to 69, and 70 and above, hypertension accounted for 2.8%, 11.6%, and 17.1% of the global disease burden, respectively, making it the leading risk factor for morbidity and mortality in individuals aged 50 and above. Hyperglycemia contributed to 2.6%, 8.4%, and 10.2% of the global disease burden across these age groups, while dyslipidemia accounted for 2.1%, 5.0%, and 4.7%, respectively.

The metabolic risk factors tend to cluster as metabolic syndrome (MetS), often defined as the presence of at least three of five metabolic risks, namely central adiposity, hypertension, hyperglycemia, high triglyceride levels, and low high-density lipid-cholesterol (HDL-C) levels.[29] MetS has been linked to an increased risk of T2DM, cardiovascular disease (CVD), and mortality, making it a valuable tool for assessing metabolic health.[30] Although there is no tracking of the global health impacts of MetS as a condition per se, a meta-analysis of 28 million individuals worldwide estimated the prevalence of MetS to range from 12.5% to 31.4% from global data published from 1990 to 2018.[31]

1.1.3 The Aging Demography and its Epidemiological Implications

The global life expectancy at birth has increased by nearly nine years since 1990, reaching 72.8 years in 2019.[4] The rise in life expectancy, in tandem with declining birth rates, is changing the population age structure worldwide.[4] By 2050, the

share of adults aged 65 years and over is projected to increase from 10% in 2022 to 16%, posing significant global challenges to the health systems.[4]

As discussed previously, the prevalence of metabolic risks increases with age, and its health impact also intensifies with age.[2, 26] As life expectancy rises, the risk of non-communicable diseases (NCDs) such as CVD, T2DM, and dementia also grows, further highlighting the global health challenges that loom ahead.[7] Although the global healthy life expectancy, an indicator of average years lived in good health, saw a modest increase of 1.4% between 2010 and 2021, it declined by 2.2% between 2019 and 2021.[7] This decline was postulated to be primarily due to the Covid-19 pandemic. Still, NCDs such as T2DM also showed significant increases in terms of disease burden during this period.[2] These trends highlight the ongoing need to prioritize addressing NCDs, which continue to rise in prevalence with age and remain a leading contributor to mortality among older adults.[2]

1.2 The BMI and the Mortality Connection

Research examining the relationship between BMI and mortality is extensive but yields inconsistent findings.[e.g.8–10, 32–36] One systematic review and meta-analysis highlights these diverse results, showing no significant association between BMI and mortality risk in the general population, while higher BMI was associated with *reduced* mortality risk in specific populations, such as individuals with CVD, Covid-19, and surgery.[33] The same study highlighted substantial heterogeneity in the numerous studies it reviewed.[33]

Despite the mixed evidence, large population-based studies at a global scale consistently revealed a nonlinear association between BMI and mortality risk, with both high and low BMI associated with increased mortality risk in the general population aged 20 to 80 years.[e.g.8, 10] This pattern of increased mortality risk associated with low and high BMI was also observed in smaller studies, including a meta-analysis of prospective cohorts of women aged 30 to 83 years,[34] and single prospective cohort studies.[9, 32, 37] A recent Mendelian randomization study has also confirmed this nonlinear pattern characterizing the BMI-mortality relationship.[38] Taken together, these studies provide solid evidence of a nonlinear BMI-mortality risk association.

Additionally, the BMI-mortality connection appears to be age-dependent. In large population-based studies that performed age-stratified analyses, the elevated mortality risk associated with high BMI attenuated among older populations.[8–10, 32,

[37] Some studies found that the BMI range associated with the lowest mortality shifted to a higher BMI level in the older age groups.[9, 32, 37]

This age-dependent pattern aligns with the findings of a comprehensive review of 71 observational cohort studies focused on individuals aged 65 and above.[35] In this review, 50% of the studies demonstrated a lower mortality risk for individuals with overweight compared to those with normal weight.[35] Furthermore, 26% of the studies reported a mortality risk reduction for individuals with obesity compared to those with normal weight [35].

In short, while substantial evidence shows that high BMI in mid-life is a risk factor for mortality, some studies suggest the presence of an 'obesity paradox,' where a higher BMI in late life may be associated with lower mortality risk.[39, 40]

1.2.1 Everyone Loves Paradoxes — About 'Obesity Paradox'

1.2.1.1 The Problem with BMI…

The obesity paradox is a phenomenon that plagues prospective and retrospective epidemiological studies concerning obesity-related health outcomes.[40-42] Although BMI is a common clinical and epidemiological measure of adiposity, it has limitations as an indicator of body fat levels.[40] These limitations may partially explain the inconsistent findings seen in studies using BMI as a tool to investigate the effects of body fat.

Firstly, BMI is calculated based on weight and height; it does not discriminate between weight attributed to fat and fat-free mass.[43] As a result, an individual classified as overweight or obese based on a BMI measure might have either a high-fat mass or a high-fat-free mass. Although this may not fully explain the obesity paradox, as fat-free mass typically decreases with aging,[44, 45] it raises concerns about using BMI in studies of a wide age range, given that body composition changes throughout adulthood.[40-42, 44, 46]

Secondly, BMI provides no information about the distribution of body fat and, thus, the location of fat depots, which are crucial for understanding metabolic health.[47] Body fat can be stored in three ways: ectopic fat, visceral adipose tissue (VAT), and subcutaneous adipose tissue (SAT) — each associated with different susceptibility to cardiometabolic morbidities.[47] High levels of VAT and ectopic fat, particularly in the liver and skeletal muscles, are associated with metabolic

dysregulation, whereas fat stored as SAT, the largest fat depot, seems more benign.[47] Since BMI cannot distinguish between these fat depots, any association between BMI and health outcomes may be influenced by varying levels of VAT, ectopic fat, or SAT. Without precise measures, it is difficult to determine the exact nature of these associations.

Unlike BMI, measurements of abdominal obesity, such as waist-hip ratios (WHR) or waist circumference (WC), are more rigorous metrics of excessive fat deposition in the abdominal region and thus better estimate VAT or ectopic fat levels. High WC in midlife is highly predictive of cardiometabolic diseases such as T2DM and CVD.[47, 48]

1.2.1.2 Additional Methodological Concerns when BMI is the exposure

The study of the BMI-mortality relationship is methodologically challenging and can be subject to biases that generate paradoxical findings, such as the obesity paradox. One potential explanation for these paradoxical findings is reverse causation, which arises when the study's outcome is the primary cause of the variations of exposure of interest. A case in point is unintentional weight loss - a consequence of comorbidities and a significant predictor of mortality.[49-51] Since low BMI may reflect unintentional weight loss in cross-sectional data, a sample population with a high prevalence of comorbid conditions that cause unintentional weight loss can fabricate spurious associations, suggesting an inverse relationship between BMI and outcomes.[52]

Reverse causation is more likely when analyses are based on a single baseline BMI measure.[53-55] Studies that rely on BMI sampled at a single time point may overlook essential aspects, which include changes in body weight over time (like unintentional weight loss just discussed), the distinct effects of BMI at various life stages (childhood, adolescence, early adulthood, midlife, and late life), and the potential for cumulative effects.[50] Investigating changes in BMI over long periods or repeated measures is crucial to better characterize the ramifications of BMI on health and avoid, control, or reduce biases from reverse causation. Nevertheless, it may not always be possible due to the lack of repeated measures.

Another potential source of bias that can fabricate a spurious inverse relationship between obesity and risk of mortality is selective survival. For

example, a study population may have a high prevalence of individuals with obesity who also have poor health, multiple comorbidities, and low survival rates. Suppose these individuals were to die before they enter into the study or are less likely to participate due to ill health; this would leave a larger proportion who are 'healthier' with obesity. In this scenario, the effects of obesity on survival may be underestimated. On this account, the obesity paradox would manifest as a result of selective survival.[52]

Factors that are associated with both the exposure (independent variable) and the outcome (dependent variable) are known as confounders. When studies do not adjust for confounders, results from statistical analysis can be biased and, thus, identify artificial relationships between variables. For example, not all studies investigating the obesity-mortality link adjust for strong confounders such as education, smoking, and cardiometabolic factors in a consistent fashion.[56, 57] Therefore, residual confounding may exist, which could, in turn, contribute to the heterogeneity in the findings, with some studies reporting a positive obesity-mortality connection while others do not.

In short, reverse causation, selective survival, and residual confounding are essential biases to consider when interpreting research on the effects of BMI.

1.2.2 Hung up or Hanging up on BMI?

The origins of BMI date back to the work of Belgian astronomer, mathematician, statistician, and sociologist Adolphe Quatelet, who, in the early 19th century, explored the relationship between height and weight to define the characteristics of an 'average man' and understand normal adult development.[58] Quatelet observed that the weight of an 'average man' generally increases proportionately to the square of height, a concept that underpins the development of BMI.[58] Several decades later, in 1972, American physiologist Ancel Keys revisited Quatelet's formula — weight divided by the square of height — and named it the "body mass index."[59] He advocated for its use as an effective tool for assessing obesity at a population level.[59]

Over the decades, BMI became a standard measure for estimating body fat levels and diagnosing overweight and obesity.[60] It continues to be the primary measure of adiposity in epidemiological studies and clinical settings to this day. BMI, calculated by dividing body weight in kilograms by the square of height in meters, categorized adults according to the World Health Organization

classification as underweight (<18.5 kg/m^2), normal weight (18.5 — 24.9 kg/m^2), overweight (25.0 — 29.9 kg/m^2), and obesity (≥30 kg/m^2).[61]

BMI is a useful tool for approximating body fat levels at the population level. Despite its limitations, the strong correlation between BMI and body fat levels observed in epidemiological studies, along with its simplicity and accessibility, have made it a widely adopted measure in both the clinical setting and at home.[62] Nonetheless, in 2023, the American Medical Association highlighted BMI's limitations in measuring body fat levels across different racial and ethnic groups, sexes, genders, and age ranges, urging the medical community to integrate alternative measures, such as visceral fat, body composition, and metabolic factors, to more accurately assess risk in clinical settings.[63]

1.3 Metabolic Health and the Mortality Connection

Robust evidence demonstrates that MetS is a significant mortality risk in the general adult population, with MetS associated with 46% and 50% higher risk for all-cause mortality from two meta-analyses.[64, 65] Unlike the association between BMI and mortality, which tends to vary with age, the connection between MetS and mortality does not appear to be age-dependent. The age-stratified meta-analysis of 21 studies found no significant difference in the all-cause mortality risk between individuals below and above 57.5 years.[64] Conversely, a meta-analysis on older populations revealed that late-life MetS was associated with a 23% higher risk in all-cause mortality.[66]

When examining individual metabolic components in the same meta-analysis among older individuals, hyperglycemia and low HDL-C levels emerged as significant mortality risk factors.[66] Conversely, higher BMI or WC paradoxically showed reduced mortality risk among older adults.[66] Hypertriglyceridemia and hypertension were not significantly associated with all-cause mortality.[66] In a separate meta-analysis that explored the relationship between MetS components and mortality among older individuals, WC and hypertriglyceridemia were not associated with increased all-cause mortality risk.[67] However, hypertension, hyperglycemia, and low HDL-C levels were found to be associated with increased mortality risk.[67]

Individual components of MetS appear to associate with mortality risk differently, with some factors showing a lack of significant associations. However, MetS as a single entity was significantly associated with mortality, raising the question of

whether MetS, a combination of its components, operates differently as a unified construct compared to its parts.

1.4 BMI feat. Metabolic Health

Although high BMI often co-occurs with other cardiometabolic risk factors, some individuals with obesity do not exhibit these abnormalities and are classified as having metabolically healthy obesity (MHO).[15–18] Conversely, while individuals with normal weight are typically metabolically healthy, some may present with metabolic dysfunction, classifying them as metabolically unhealthy normal weight (MUN), sometimes referred to as 'thin on the outside, fat on the inside,' abbreviated as TOFI.[68]

A meta-analysis of 43 cohort studies encompassing 4.8 million individuals across all BMI categories found that the prevalence of MHO ranged from 1.2 — 31%, with a median of 6.6%.[20] In addition, studies have reported that the prevalence of MUN ranged from 18% to 56%.[69, 70] These findings indicate significant heterogeneity within each BMI classification. By incorporating metabolic health profiles, individuals can be better characterized beyond BMI alone, as demonstrated in Figure 2, which illustrates the diverse range of metabolic health-BMI phenotypes.

The concepts of MHO and MUN underscore the heterogeneity within BMI categories.[71] These findings call for a re-evaluation of whether BMI alone is sufficient for understanding the development of cardiometabolic morbidities. It raises a critical question: is the increase in body fat a cause or the consequence of cardiometabolic dysfunction? Without a definitive answer, it may remain crucial to consider body fat levels in conjunction with other cardiometabolic components to enhance our understanding of their impact on human health.

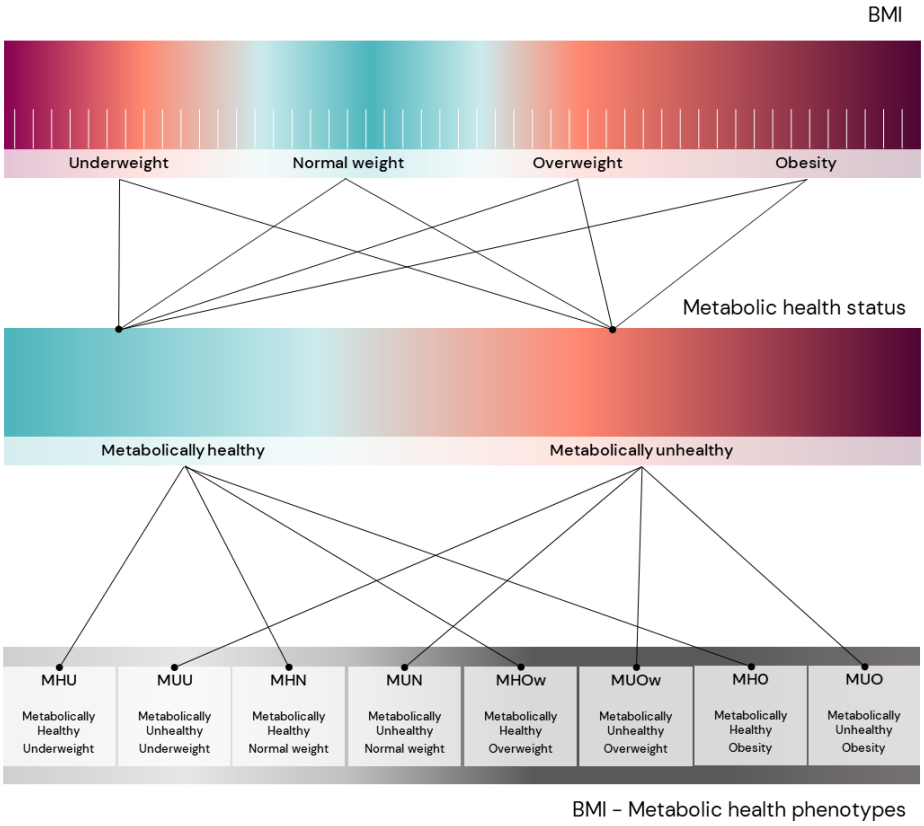

Figure 2: BMI-metabolic phenotypes from the cross-categorizations of BMI and metabolic health

Figure illustrates the cross-categorization of individuals based on their BMI and metabolic health status, resulting in various BMI-metabolic health phenotypes. The top bar represents the BMI spectrum that can be on a continuous scale or categorized into underweight, normal weight, overweight, and obesity. Middle bar represents metabolic health status that can be divided into metabolically healthy and unhealthy. The bottom bar depicts the possible BMI-metabolic health phenotypes from the combination of BMI and metabolic health categories.

Abbreviations: BMI – body mass index

1.4.1 Defining Metabolic Health and Metabolically Healthy Obesity

There is no standardized definition of metabolic health, which leads to marked variability in the classification of MHO.[15, 72] Typically, MHO is defined by adapting the criteria for MetS, where the absence of MetS indicates a metabolically healthy state.[73] As a result, MHO is commonly characterized as having obesity (BMI ≥ 30 kg/m²) without the manifestation of MetS.[15] However, different definitions of MetS exist, as Table 1 outlines the four most commonly used in the literature.[29, 74-77]

Table 1: Different criteria for ascertaining metabolic syndrome

	NCEP ATP III – 2009 [29]	WHO – 1998[74]	EGIR – 1999[75]	IDF – 2005[76]
Compulsory requirements	None	• IR, • Impaired glucose tolerance, • Impaired fasting glucose, • T2DM diagnosis, or • Other evidence of IR	• IR • plasma insulin > 75th percentile for patients without T2DM	• Central obesity determined by WC: • ≥ 94 cm in M, • ≥ 80 cm in F
Criteria	Any 3 of the 5 below	Plus 2 of the 5 below	Plus 2 of the 4 below	Plus 2 of the 4 below
*Obesity or central obesity	WC • ≥80 cm in F, • ≥94 cm in M	WHR • >0.90 in M, • >0.85 in F, or • BMI ≥ 30 kg/m2	WC • ≥80 cm in F, • ≥94 cm in M	Compulsory component defined above
Hyperglycemia	• Fasting glucose ≥100 mg/dl, or • Treatment, or • T2DM diagnosis	Compulsory component defined above	Fasting glucose ≥ 6.1 mmol/l for nondiabetic	• Fasting glucose ≥ 100 mg/dl, or • T2DM Diagnosis
Dyslipidemia based on plasma TG levels	• TG ≥150 mg/dL (1.7mmol/L), or • Treatment	• TG ≥ 150 mg/dl or • HDL-C <35 mg/dL in M, <39 mg/dL in F • Treatment	• TG > 180mg/dl, or • HDL-C < 40mg/dl, or • Treatment	• TG > 150mg/dl, or • Treatment
Dyslipidemia based on HDL-C levels	• <40 mg/dl in M, • <50 mg/dl in F, or • Treatment	Combined with TG above	Combined with TG above	• < 40 mg/dl in M, • <50 mg/dl in F • Treatment
Hypertension	• Systolic blood pressure ≥ 130 mmHg and • Diastolic blood pressure ≥ 85 mmHg, or • Treatment	≥ 160/90 mmHg	• ≥140/90 mmHg or Treatment	• Systolic ≥ 130mmHg or • Diastolic ≥ 85 mmHg or • Treatment

Continue

	NCEP ATP III – 2005 revision [29]	WHO – 1998 [74]	EGIR – 1999 [75]	IDF – 2005 [76]
Albumin levels	Not included	• Microalbuminuria, or • Urinary albumin excretion ≥20 ug/min, or • A:C ≥ 20mg/g	Not included	Not included

*Indicates threshold values in this table were based on the European population.

Abbreviations: NCEP ATP III - National Cholesterol Education Program Adult Treatment Panel-III; WHO – World Health Organization; EGIR – European Group for the Study of Insulin Resistance; IDF – International Diabetes Definition; T2DM – Type II Diabetes Mellitus; BMI – body mass index, IR – Insulin resistance, WC – waist circumference; WHR – waist-hip-ratio; M – males; F – females; TG – triglyceride levels: HDL-C – high-density lipoprotein cholesterol, A:C – albumin to creatinine ratio

Some argue that being truly metabolically healthy should entail the absence of any metabolic abnormalities.[15] This has led to two approaches for defining metabolically healthy states in MHO: a stricter definition, which requires the complete absence of any metabolic deficiencies, and a less strict definition, which defines metabolic health as the absence of MetS, typically allowing for no more than one metabolic abnormality.[15, 72]

Several authors have called attention to the need for consensus in the definition of metabolic health to advance research in this area.[15, 16, 71, 78] Zembic et al. initiated an attempt to define the MHO phenotype systematically.[79] They identified an MHO phenotype associated with no increased risk of CVD mortality and all-cause mortality, while all unhealthy groups showed substantially increased risk.[79] According to their study, metabolic unhealthy status was based on three components: hypertension from systolic blood pressure, increased waist-hip ratio, and prevalent diabetes.[79] These newly established criteria will need further validation in other cohorts to ensure their applicability and accuracy.

1.4.2 Skinny on Metabolically Healthy Obesity

MHO is distinguished by biological mechanisms and phenotypes that diverge from those associated with metabolically unhealthy obesity (MUO).[15] MHO has lower levels of ectopic fat and VAT, increased SAT and leg fat levels, insulin sensitivity, normal leptin activity, normal levels of inflammatory markers, and

normal adipose tissue function, compared to MUO.[15, 47] In individuals with MHO, adipose tissue tends to grow by cell quantity, resulting in less inflammation. In contrast, adipose tissue in MUO typically grows by increasing the cellular size instead of quantity, leading to more inflammation.[15, 47]

The prognosis of the MHO phenotype is still debated, as past research yielded heterogeneous results. A meta-analysis of prospective cohort studies has reported that compared to MHN, MHO significantly increased the risk of all-cause mortality by 59%.[22] MHO was also associated with an increased risk of cardiovascular mortality, although the findings were not statistically significant.[22] The metabolically healthy overweight MHOw phenotype significantly increased the risk of all-cause and cardiovascular mortality by 22% and 34%, respectively, compared to MHN.[22]

Most research on MHO has focused on relatively younger adults, with the mean age of under 55 and the mean follow-up periods ranging from 3.6 to 30 years.[22] Limited research investigates the BMI-metabolic health phenotypes and mortality among older persons. One study, which included 4551 participants aged 67 to 74 with a mean follow-up of 10.9 years, found that MHOw and metabolically healthy class I obesity(BMI between 30 to 35 kg/m^2) had a 10% and 42% lower mortality risk relative to MHN, respectively.[80] In contrast, metabolically unhealthy class II obesity (BMI ≥ $35kg/m^2$) and metabolically unhealthy normal weight phenotypes were associated with increased mortality risk.[80] Even when BMI was examined in conjunction with metabolic health, the evidence suggested the better prognosis with higher BMI among older individuals persisted as long as they were metabolically healthy.[80]

A significant limitation of current literature on MHO is that most studies have assessed BMI-metabolic health phenotypes at a single time point. Nevertheless, several authors have posited that MHO and other BMI-metabolic health phenotypes may be transient.[15, 29, 47, 69, 71, 81] The largest study to date, which followed 90,257 women over 30 years, reported that 84% of women classified as MHO transitioned to a metabolically unhealthy state over time.[81] Although those who remained in the MHO state were associated with a higher risk of CVD compared to women with stable, healthy, normal weight, their risks were lower than those who transitioned from a healthy to an unhealthy metabolic state, regardless of BMI category. Despite these findings, there is limited knowledge of the longitudinal

transitions in BMI-metabolic health phenotypes and their effects on morbidity and mortality. These are critical areas for future research to address.

1.5 Biological Aging Simplified

Chronological age is the number of years since an individual's birth. Underlying this definition of age is the assumption that the passing of each unit of time (days, weeks, months, or years) is uniform for everyone, which is not plausible. In contrast to chronological age, biological age captures heterogeneity in aging among individuals and can be measured by quantifying various biomarkers of aging.[23] These biomarker measures can provide a deeper understanding of the aging process and overall health. They can be valuable for examining the connections among BMI, metabolic health, and BMI-metabolic health phenotypes, aging, and overall health outcomes.

Biological age measures (BA) can be broadly categorized into functional, physiological, and cellular levels, each reflecting changes during aging at these hierarchical levels of biological organization.[82]

1.5.1 Biological Aging at the Functional Level

Developing and maintaining functional ability is vital to healthy aging.[83] Metrics that assess aging at the functional level provide valuable insights into overall health during aging.[84] These measures may capture age-related changes in physical, cognitive, emotional, and mental domains.[82] The functional aging index (FAI) is an example derived from four components: gait speed, grip strength, peak expiratory flow, and subjective sensory ability.[84] The FAI is a composite score such that a higher FAI indicates greater biological aging.[84] This means that as FAI increases, it reflects higher levels of decline in physical and sensory functions, suggesting that the individual is experiencing more pronounced aging effects across these functional domains.[84]

1.5.2 Biological Aging at the Physiological Level

At the physiological level, biological aging reflects changes in body systems, such as metabolism and cardiovascular function, which often contribute to the development of diseases.[82] The frailty index (FI) is a biological age measure that captures physiological and functional changes.[85] Developed by Rockwood, FI is a ratio calculated as the number of health deficits present over the total number of health deficits considered.[86] The health deficits considered within FI are diverse

and include symptoms like fatigue and pain, clinical signs such as hypertension and hyperglycemia, the presence of chronic diseases such as T2DM, physical disabilities, and results from laboratory tests, radiographic imaging, and electrocardiographic evaluations.[86] Generally, 40 different deficits are considered in FI, and a minimum of 30 is necessary to maintain its predictive value for the risk of adverse events.[86]

1.5.3 Biological Aging at the Epigenetic Level

The programmed theory of aging posits that aging is regulated by specific mechanisms akin to a biological clock, which systematically controls the aging process.[87] This theory postulates that aging is not the primary purpose of these regulatory mechanisms but rather an unintended consequence of biological functions and programmatic processes.[87] At the cellular level, the concept of epigenetic clock, based on the methylation pattern of deoxyribonucleic acid (DNA), has gained prominence as a tool to understand these processes.[88]

The earliest epigenetic clocks were developed using statistical models that were trained to predict chronological age based on a weighted average of DNA methylation (addition of a methyl group) levels at combinations of CpG sites — regions of DNA where a cytosine nucleotide is positioned before a guanine nucleotide.[23, 89] The resultant DNA-methylation measure, known either as DNA methylation age or epigenetic age, correlates strongly with chronological age.[23, 89] Deviations of epigenetic age from the chronological age, known as epigenetic age acceleration (EAA), can predict age-related morbidities and mortality.[90] The four most commonly used are Horvath,[89] Hannum,[91] PhenoAge,[92] GrimAge,[93] and PACE (see Table 2 for comparisons).[94]

Horvath's[89] and Hannum's[91] clocks were constructed based on CpG sites correlated with chronological age, while PhenoAge incorporates chronological age and nine clinical biomarkers to predict better lifespan and health span.[92] GrimAge, described by its inventors as a 'biomarker of mortality,' was derived by selecting CpG sites associated with smoking-pack-years and plasma proteins, followed by further regression of time-to-death on these chosen biomarkers.[93] PACE, on the other hand, measures the pace of aging by examining changes in 19 biomarkers over 12 years of follow-up.[93]

Table 2: Comparisons of epigenetic clocks

Epigenetic clock, year	N. of CpGs	Source of DNA	Training targets
Horvath, 2013[89]	353	Multiple tissues, including blood	Chronological age
Horvath, 2018[95]	391	Skin, blood, and saliva samples	Chronological age
Hannum, 2013[91]	71	Whole blood, leukocytes	Chronological age
PhenoAge, 2018[92]	513	Whole blood	Chronological age + 9 biomarkers: *albumin, creatinine, serum glucose, C-reactive protein, lymphocyte percent, mean red cell volume, red cell distribution width, alkaline phosphatase, white blood cell count*
GrimAge, 2019[93]	1030	Whole blood	Chronological age + sex + smoking pack-years + 12 plasma proteins: *adrenomedullin, beta-2-microglobulin, CD56, ceruloplasmin, cystatin-C, EGF fibulin-like ECM protein 1, growth differentiation factor 15, leptin, myoglobin, plasminogen activator inhibitor, serum paraoxonase/arylesterase 1, tissue inhibitor metalloproteinases 1*
PACE, 2022[94]	46	Whole blood	Chronological age + sex + longitudinal change of 19 biomarkers: *BMI, waist-hip ratio, glycated hemoglobin, leptin, mean arterial pressure, cardiorespiratory fitness (VO_2Max), forced expiratory volume in one second (FEV_1), FEV_1/forced vital capacity, total cholesterol, triglycerides, high-density lipoprotein, lipoprotein(a), apolipoprotein B100/A1 ratio, estimated glomerular filtration rate (eGFR), blood urea nitrogen (BUN), high sensitivity C-reactive protein, white blood cell count, mean periodontal attachment loss, the number of dental-caries-affected tooth surfaces*

Abbreviations: N. - number

1.5.4 Connecting BMI and Metabolic Health with Biological Aging

1.5.4.1 Associations with Functional and Physiological Aging

A meta-analysis of 12 studies involving 74,985 individuals above the age of 60 found that underweight and obesity were associated with a statistically significant increase in the risk of frailty (defined as FI≥ 21%, or the presence of three of the following: involuntary weight loss, exhaustion, slow gait speed, poor handgrip strength, and sedentary behavior), by 45% and 40%, respectively, compared to

normal weight. In contrast, overweight was associated with a 7% reduced risk of frailty; however, this estimate did not reach statistical significance.[96] Additionally, abdominal obesity was reported to increase the risk of frailty by 57% based on data from 6 studies that included a total of 18,764 individuals.[96] These findings highlight that both lower and higher BMI may impact overall health outcomes during aging.

Similarly, MetS has also been linked to an increased risk of frailty, indicating the crucial role metabolic health may play in health outcomes during aging. A meta-analysis reported a 73% increased risk of frailty among those with MetS relative to those without MetS.[97] Moreover, a cross-sectional study using data from the US National Health and Nutrition Examination Survey found a positive correlation between MetS and FI in 6,403 participants aged below 65 years, which was attenuated in 2,152 participants aged 65 years or older, suggesting age-dependent effects between MetS and FI.[98] Together, these findings emphasize the complex interplay between BMI, metabolic health, and biological aging at the physiological level.

The FAI, being a relatively new BA, has not been studied extensively. There is, however, a moderate correlation between FAI and FI (correlation coefficient of 0.46).[84] FAI and FI seem to capture different facets of the aging process, suggesting they may be complementary.[84]

1.5.4.2 Associations with Epigenetic Aging

Higher BMI is consistently linked to increased epigenetic age.[99] Nevertheless, research reveals that this association may differ across various tissue types.[99] For instance, elevated BMI has been associated with higher EAA in the liver tissue[100] and VAT,[101] but not in whole blood. In contrast, several other studies have consistently found that higher BMI correlates with higher EAA in whole blood.[102-106]

Age-stratified analyses highlight that the relationship between BMI and EAA may differ across age groups. Specifically, one study found a positive correlation between BMI and EAA in middle-aged individuals but not in older adults.[107]

Most of these studies that examined the BMI-EAA association referenced above are cross-sectional in design, which limits the ability to determine the direction of the BMI-EAA association. Therefore, the direction of the association remains elusive. However, a recent Mendelian randomization study revealed a bidirectional association between obesity and EAA, with more substantial effect sizes observed

when obesity leads to increased EAA.[108] These findings underscore the necessity for research to elucidate further the pathways underlying the BMI-EAA relationship.

Research investigating the relationship between metabolic health and EAA has identified significant associations. A longitudinal study showed that poor cardiovascular health (based on a composite score derived from BMI, blood pressure, glucose, and cholesterol) in early adulthood is associated with higher EAA 15 and 20 years later.[109] Although only a few studies have specifically explored the connection between MetS and the EAA, the existing evidence generally indicates that MetS is associated with higher EAA.[93, 102, 110-113]

Similar to the associations between BMI and EAA, there are suggestions of age-specific effects in the MetS-EAA relationship. For example, a study based on a Korean population found a positive correlation between MetS and the acceleration of GrimAge among middle-aged but not older participants.[114] Furthermore, a recent twin study showed that accelerated epigenetic age was linked to MetS independently of lifestyle factors, with genetics contributing to this connection.[113]

1.6 When Obesity and Aging Collide

Aging and obesity, though distinct conditions, may be intricately connected. Aging, often perceived as an innate and inevitable process, involves the gradual accumulation of perturbations to physiological functions that increase vulnerability to diseases and mortality.[115] According to the geroscience hypothesis, aging can be accelerated or decelerated, depending on genetic, environmental, and lifestyle factors.[116] In contrast, obesity is a chronic, relapsing disease characterized by excessive accumulation of body fat.[47] Its pathogenesis is multifactorial, involving complex interactions between genetic predispositions, tissue dysfunction, hormonal imbalances, and environmental factors such as lifestyle and diet.[47] Both conditions share the potential for modulation, shaping their progression by the complex interplay of biological and external factors.

Despite their differences, aging and obesity share many similarities in their pathophysiologies. While a thorough exploration of these similarities is beyond the scope of this thesis, key parallels have been reviewed by Tam et al.[117] and Diaz-Ruiz et al.[118] For example, both aging and obesity are associated with increased oxidative stress caused by the overproduction of reactive oxygen

species (ROS). This oxidative stress damages cellular structures such as DNA and proteins, leading to tissue damage.[117, 118] In both conditions, prolonged exposure to high levels of ROS can damage mitochondrial DNA, resulting in mitochondrial dysfunction.[115] This dysfunction, in turn, contributes to chronic systemic inflammation and cellular senescence, which are well-established hallmarks of aging.[115] Thus, the mechanisms seen in obesity not only mirror those of aging but also actively accelerate the aging process.

Insulin resistance is a central pathophysiological mechanism that drives metabolic dysfunction in aging and obesity. In aging, increased visceral fat, senescent cells, and inflammatory cytokines from chronic inflammation likely impair insulin signaling pathways, leading to insulin resistance.[119] In obesity, a combination of genetic and environmental factors can lead to hyperinsulinemia and, eventually, to insulin resistance, which impairs the glucose uptake by the cells, further promoting fat storage and metabolic deterioration.[47] These shared mechanisms — oxidative stress, chronic inflammation, mitochondrial dysfunction, and insulin resistance — may drive the development of diseases, such as T2DM, CVD, and neurodegenerative conditions in both aging and obesity.[117-119]

1.7 Minding the Gaps

A vast body of literature explores the relationships between BMI, metabolic health, and aging, yet critical gaps remain in fully understanding the complexities of how these factors interact.

One significant gap involves the "obesity paradox," which suggests that higher BMI in older adults may be linked to better health outcomes, contrary to the established risks in younger populations. This paradox may stem from the limitations of BMI as a measure and methodological challenges such as reverse causality and selective survival when studying BMI-mortality associations. To address these limitations, using biological aging metrics as markers of late-life health could provide valuable insights. Biological aging measures capture the heterogeneity of aging processes and offer a more comprehensive view of late-life health. This could help clarify the potential impact of BMI and metabolic health in late-life health.

Another critical gap is the ambiguity surrounding the concept of metabolic health, including its definition and influence on health outcomes across the BMI spectrum. There is no universally accepted definition of metabolic health, and various studies use different criteria, leading to inconsistent conclusions. Furthermore, much-existing research considers BMI and metabolic health independently, while their joint effects on aging — especially biological aging — are poorly understood. The concept of "metabolically healthy obesity" exemplifies this uncertainty. It remains unclear whether MHO represents a genuinely lower-risk form of obesity or if it eventually leads to increased health risks, particularly in older adults. Understanding the joint effects of BMI and metabolic health and the implication of MHO is essential for better characterizing late-life health risks associated with BMI and metabolic health.

The overlapping pathophysiologies of obesity and biological aging suggest that obesity may accelerate the aging process, yet the directionality and dynamics of this relationship remain unclear. The roles of biological aging, obesity, and metabolic health in determining mortality, particularly in older adults, have not been fully explored. Investigating how each factor contributes to survival outcomes could reveal important mechanisms driving health outcomes in aging populations.

2 Research Aims

This thesis aims to clarify the complex relationships between BMI, metabolic health, and aging while addressing research gaps as best as we can and considering the non-linearity of BMI and age-specific effects. Specifically, we focused on how BMI and metabolic health are associated with biological aging and mortality risk. The thesis was structured around four specific studies, each with its objective:

Study I: To examine the independent and joint associations of BMI and metabolic health in midlife and late life with all-cause mortality.

Study II: To assess the cross-sectional association between BMI, metabolic health, and biological aging, using the functional aging index and frailty index as markers of aging at the functional and physiological levels.

Study III: To investigate the role of biological aging, as measured by epigenetic age acceleration, in mediating the relationship between BMI and mortality.

Study IV: To explore the longitudinal association between BMI and biological aging, focusing on how changes in BMI influence biological aging and how changes in biological aging itself may drive changes in BMI over time.

3 Materials and Methods

3.1 Overview of Data Sources

This thesis drew data from several population-based cohorts, including four sub-studies of aging within the Swedish Twin Registry (STR):[120] Aging in Women and Men: A Longitudinal Study of Gender Differences in Health Behaviour and Health among Elderly (GENDER),[121] the Origins of Variances in the Oldest-Old: Octogenarian Twins (OCTO-Twin),[122] the Swedish Adoption/Twin Study of Aging (SATSA),[123] and TwinGene.[124] The vital status of the participants within each sub-study was obtained through linkages to several nationwide registries. In addition, data from the Health and Retirement Study (HRS) in the U.S. was also utilized.[125]

3.1.1 Aging in Women and Men: A Longitudinal Study of Gender Differences in Health Behaviour and Health among Elderly

GENDER is a longitudinal study involving data collection through mail-in surveys and in-person testing and interviews (IPT) between 1994 and 2007.[121] It constitutes Swedish twin pairs of the opposite sex born between 1906 and 1925 and alive in 1994. A baseline mail-in survey was performed in 1994, and information on demographics, health status, health-related behaviors, and psychosocial aspects was collected. A total of 1,843 individuals responded to the survey, translating to a 54% individual response rate.[121] Then, a selected sample underwent three waves of IPTs at approximately four-year intervals. The first IPT, which took place from 1995 to 1997, included 498 twins from 249 twin pairs of opposite sex with a mean age of 74.6 years [standard deviation (SD) = 2.6) at that time.[126] Two additional IPTs were performed from 1999 to 2001 and 2003 to 2005. Each IPT involved interviews, cognitive tests, physical functioning tests, health examinations, and blood sampling conducted by trained healthcare professionals. The last survey was mailed in 2007 to all living participants.

3.1.2 Origin of Variances in the Oldest-Old: Octogenarian Twins

OCTO-Twin is a longitudinal study of Swedish same-sex twin pairs focused on twins aged 80 and above.[122] Initiated in 1991, OCTO-Twin conducted five IPTs until 2002 at two-year intervals.[127] A total of 1,098 twins over 79 were invited to participate, with 702 twins (351 complete twin pairs) accepting the invitation and participating in the first IPT, yielding a participation rate of 64%.[128] The mean age

at the first IPT was 83. (SD = 3.17).[127] Each IPT was conducted by licensed nurses at the participant's residence, who collected data on participants' socio-demographics, life histories, health-related behaviors, and blood samples.[128] Participants also underwent comprehensive cognitive tests, physical functioning assessments, and health examinations.[122]

3.1.3 Swedish Adoption/Twin Study of Aging

SATSA is a comprehensive longitudinal study that includes twins reared apart before age 11 and matched control pairs of twins reared together, matched according to sex, date of birth, and country of birth.[123, 129] Initiated in 1984, SATSA is the earliest of the aging sub-studies compared to GENDER and OCTO-Twin, and it continued until 2014, spanning 30 years.[123] Data for SATSA were obtained through mail-out questionnaires and IPTs at approximately three-year intervals.[123] The study began with the first questionnaire sent in 1984 to 2,854 individuals aged 50 and above.[123] A total of 2,019 individuals responded (71%).[123] Among those who responded were 758 complete twin pairs, of which 351 twin pairs were reared apart before age 11 and 407 control pairs of twins reared together.[123]

The questionnaires in SATSA consist of self-reported data on physical and mental health, personality traits, health behaviors, and environmental factors. At the same time, IPT involves biomedical assessments, physical functioning tests, and cognitive evaluations conducted by registered nurses.[123] A total of 645 individuals, with a mean age of 63.6 (SD=8.8), participated in the first IPT in 1986. By the study's conclusion, in 2014, nine questionnaires and ten IPTs had been completed, with 859 twins participating in at least one IPT.[129]

3.1.4 TwinGene

TwinGene is a cross-sectional study involving Swedish twins born between 1911 and 1958 who had previously participated in the Screening Across the Lifespan Twin (SALT) study conducted from 1998 to 2002.[124] Data collection for TwinGene occurred between 2004 and 2008 through a health questionnaire, which focused on inquiries on CVD, T2DM, and medical interventions such as surgeries and medication use.[124] Participants also underwent a health examination and provided blood samples at local healthcare facilities.[124] Of the 22,391 twins invited, around 14,600 (65%) responded to the questionnaire, and 12,614

individuals (56%), including 5,014 complete twin pairs, completed the health examination.[124]

3.1.5 The Health and Retirement Study

The HRS is a large-scale, longitudinal study designed to explore the factors influencing aging in a nationally representative sample of U.S. residents aged 50 and over.[125] Since its launch in 1992, HRS has collected data every two years on various topics, including health, financial status, cognitive abilities, employment, retirement, and family dynamics.[125] The first data collection primarily involved face-to-face interviews, with follow-up interviews conducted via telephone — except for participants aged 80 and above, who continued to have in-person interviews.[125]

HRS broadened its scope to incorporate biological markers, genetic data, and more detailed psychological and social measures in 2006.[125] As part of this expansion, half of the participants underwent face-to-face interviews, which included the collection of biomarkers, while the other half continued with telephone interviews only.[125] These face-to-face interviews, along with the collection of biomarkers (dried blood spots), alternated between halves of the sample, ensuring that comprehensive data was gathered for each participant every four years.[125] Additionally, HRS is linked to administrative records such as Social Security, Medicare, and the National Death Index, providing a rich dataset for examining the impact of aging on individuals and broader population trends.[125]

The initial wave in 1992 included 15,497 invited participants, with 12,652 completing interviews, resulting in a strong response rate of 81.6%.[125] Over time, additional cohorts were incorporated, including the Asset and Health Dynamics Among the Oldest Old (AHEAD) in 1993, the Children of the Depression (CODA) and War Babies in 1998, Early Baby Boomers in 2004, and Mid Baby Boomers in 2010.[125] Although response rates have gradually declined, HRS has surveyed over 37,000 individuals from more than 23,000 households.[125] Between 1992 and 2020, the number of individuals contacted in each wave ranged from 7,555 to 27,198, with respondents per wave ranging from 7,027 to 22,032.[130] The latest wave of data collection occurred in 2022.

3.1.6 Data Source Allocation Across Studies

Figure 3 shows the data collection timelines for each cohort and the specific data sources used in each study.

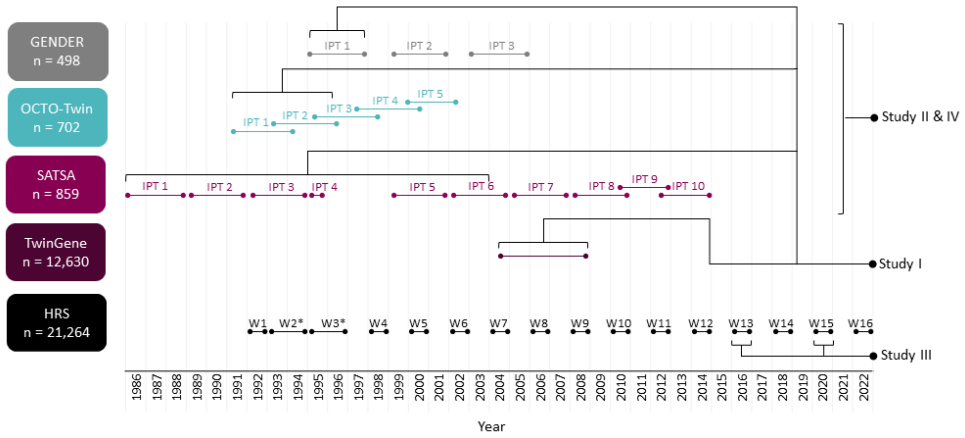

Figure 3: Data collection timelines for each cohort and study-specific data sources.

Abbreviations: IPT – in-person testing, W – data collection wave

Study I aimed to examine how BMI and metabolic health in midlife and late life are independently and jointly associated with mortality risk. This study used pooled data from IPT1 of the GENDER study, IPT1-3 of OCTO-Twin, IPT1-6 of SATSA, and TwinGene.

The vital status of participants as of December 31, 2020, was obtained from the Swedish Tax Register.

Study II, which assessed the cross-sectional associations between BMI, metabolic health, and biological aging, and **Study IV**, which explored the longitudinal relationship between BMI and biological aging, utilized data from all IPTs across GENDER, OCTO-Twin, and SATSA.

Study III investigated the mediating role of epigenetic aging in the relationship between BMI and mortality risk while adjusting for metabolic health. The analysis used data from the 2016 HRS when the Venous Blood Study (VBS) was introduced. All respondents from the 2016 wave were invited to participate in VBS, with 78.5%

accepting the invitation and 82.9% completing blood sampling, resulting in a total sample size of 9,934 individuals.[131] The venous blood samples collected were assayed for numerous aging markers, many of which were metabolic, enabling the inclusion of metabolic health variables in **Study III**. From this group, a non-random subsample of 4,104 blood samples underwent DNA methylation assays, from which epigenetic age measures were derived and used in this analysis.[132] Data on participants' vital status as of December 2020 was also incorporated.

3.2 Operationalization of Study Variables

3.2.1 Body Mass Index

BMI was calculated by dividing weight in kilograms (kg) by the square of height in meters (m^2). As a categorical variable (**Study I**), BMI was classified according to the WHO criteria as underweight (<18.5kg/m^2), normal weight (18.5 — 24.9 kg/m^2), overweight (25.0 — 29.9 kg/m^2), and obesity (≥30kg/m^2).[61] The remaining studies treated BMI as a continuous variable (**Study II–IV**).

In GENDER, OCTO-Twin, SATSA, and TwinGene, BMI data were obtained from measurements of height and weight taken by trained healthcare professionals. During the measurements, participants were instructed to remove their shoes, heavy items, and bulky clothing.

BMI data from the HRS study was based on self-reported height and weight during telephone interviews. From 1998, height was only recorded during the first interview and then carried forward in subsequent survey waves.

3.2.2 Metabolic Health

The operationalization of metabolic health status (MHS) across the studies was based mainly on the National Cholesterol Education Program Adult Treatment Panel-III (NCEP ATP-III) criteria for MetS,[29] along with insights from previous research[15, 72] and data availability from each cohort. Since no consensus exists on the definition of metabolic health,[15] we applied the most appropriate definitions using all available data. This thesis typically considered the following metabolic components: hypertension, hyperglycemia, hypertriglyceridemia, and low HDL-C levels.

While the NCEP ATP-III criteria also included central obesity, the primary analyses excluded this factor. However, central obesity was incorporated into the definition of metabolic health in sensitivity analyses when measures were available.

According to NCEP ATP-III criteria, MetS is defined as the presence of three or more of the five metabolic components: hypertension, hyperglycemia, hypertriglyceridemia, low HDL-C, and central obesity. In primary analyses that excluded measures of central obesity, metabolically unhealthy status was defined by the presence of two or more metabolic components (less strict definition) or one or more components (strict definition). Table 3 provides the specific criteria used by the data source and study.

3.2.3 Hypertension

Hypertension was ascertained in GENDER, OCTO-Twin, SATSA, and TwinGene through systolic and diastolic blood pressure measured by licensed healthcare professionals. In GENDER and TwinGene, participants' systolic and diastolic blood pressure was measured while seated, whereas in OCTO-Twin and SATSA, measurements were taken with participants supine. In all three studies, measurements were taken after five minutes of rest, followed by a second reading after an approximately one-minute pause, with the lower of the two readings recorded. Blood pressure was not measured in the HRS VBS study, so hypertension was identified based on participants' self-reports of a prior diagnosis from a doctor indicating high blood pressure or hypertension.

3.2.4 Hyperglycemia

Based on available data, hyperglycemia was determined using a combination of venous blood glucose levels (fasting and non-fasting), hemoglobin A1c (HbA1c) levels, self-reported use of T2DM medications, and self-reported T2DM diagnosis. In SATSA, venous blood glucose levels were available and used to define hyperglycemia. Venous blood HbA1c levels were used for this purpose in TwinGene. All Swedish twin cohorts provided self-reported information on T2DM diagnosis and medication use, which were used to ascertain hyperglycemia. For the HRS cohort, hyperglycemia was determined using both venous blood glucose levels and self-reported T2DM diagnosis.

Table 3: Operationalization of metabolic health by cohort and study

		Data sources					Studies in thesis		
Metabolic component	**Diagnostic criteria**	**GENDER**	**OCTO-Twin**	**SATSA**	**TwinGene**	**HRS/VBS**	**Study I**	**Study II**	**Study III**
Hypertension	Systolic BP ≥ 130 mmHg	X	X	X	X		X	X	
	Diastolic BP ≥ 85 mmHg	X	X	X	X		X	X	
	Self-reported hypertension					X			X
Hyperglycemia	Fasting BG ≥ 6.1 mmol/L (100 mg/dL)			X		X	X	X	X
	Non-fasting BG ≥ 7.0 mmol/L (125 mg/dL)			X		X	X	X	X
	HbA1c ≥ 5.7%				X		X		
	Self-reported use of diabetic medications	X	X	X	X		X	X	
	Self-reported diagnosis of type II diabetes	X	X	X[§]	X	X	X	X	X
Low HDL-C	< 1.03 mmol/L (40 mg/dL) in males or < 1.30 mmol/L (50 mg/dL) in females	X*	X[┼]	X	X	X	X	X	X
	Self-reported use of lipid-lowering medications	X	X	X	X		X	X	
Hypertriglyceridemia	Fasting TG ≥ 1.70 mmol/L (150 mg/dL)	X[ǂ]		X	X	X	X	X	X
	Non-fasting TG ≥ 2.1 mmol/L (186 mg/dL)	X[ǂ]		X	X	X	X	X	X
	Self-reported use of lipid-lowering medications	X	X		X		X	X	
Central Obesity	Waist circumference >94 cm in males or >80 cm in females	X	X	X	X		X		
	Waist-hip-ratios >0.90 in males or >0.80 in females	X	X	X	x		X[P]	X	
Less strict MU	≥2 metabolic components						Main	Main	Sens.
Strict MU	≥1 metabolic component						Sens.		Main
MetS (considered MU)	≥3 metabolic components (including central obesity)						Sens.	Sens.	

An 'x' in the cell indicates the criteria used to determine the presence of each metabolic component. Metabolic components were considered present if any one of the diagnostic criteria was met. * indicates measures available only in IPT3, and ǂ indicates measures available in IPT1 and IPT3 in GENDER. ┼ indicates measures available only in IPT2 in OCTO-Twin. 'Main' refers to the definition of metabolically unhealthy status used in the primary analysis, while 'Sens.' refers to the definition used in sensitivity analyses. [P] indicates a different cut-off for waist-hip-ratio was used (≥1.03 in males or ≥0.95 in females based on Zembic et al.[79]).

Abbreviations: BP – blood pressure, BG – blood glucose levels from venous blood samples, HbA1c – hemoglobin A1c percentage from venous blood samples, TG – triglyceride level from venous blood samples, HDL-C – high-density lipoprotein cholesterol from venous blood samples, MU – metabolically unhealthy status, MetS – metabolic syndrome, Sens. – sensitivity analyses

3.2.5 Hypertriglyceridemia

Hypertriglyceridemia was determined using venous blood triglyceride levels (in both fasting and non-fasting conditions) and self-reported use of lipid-lowering medications in GENDER, SATSA, and TwinGene. Since triglyceride levels were not measured in OCTO-Twin, hypertriglyceridemia in this cohort was based solely on self-reported lipid-lowering medication use. In the HRS VBS study, hypertriglyceridemia was ascertained through venous blood triglyceride levels.

3.2.6 Low HDL-C

Low HDL-C was determined using venous blood HDL-C levels, available across all cohorts. In addition, the Swedish Twin cohorts also considered self-reported use of lipid-lowering medications as part of the criteria for identifying low HDL-C status.

3.3 Biological Aging Measures

3.3.1 Functional Aging Index

FAI is used to quantify biological aging at the functional level. It is a composite score derived from three physical performance metrics: gait speed, grip strength, peak expiratory flow (PEF), and self-reported sensory ability.[84] A higher FAI score reflects more advanced aging in the functional domain. Gait speed was measured as the time taken to walk 3 meters and back, while grip strength was determined by recording the highest of three attempts from each hand using a dynamometer or vigorimeter.[84] PEF was calculated as the best result from two trials using a portable spirometer.[84] Licensed healthcare professionals in GENDER, OCTO-Twin, and SATSA.83 measured the functional metrics, gait speed, grip strength, and PEF.

Vision and hearing were assessed through self-reported measures.[84] In SATSA, participants rated their vision on a scale from 1 (excellent) to 5 (nearly blind or blind) and their hearing from 1 (excellent) to 5 (nearly deaf or deaf).[84] The two scores were combined to form a single measure of subjective sensory ability.[84] OCTO-Twin and GENDER used 6-point scales to rate vision and heating; therefore, the responses were adjusted to a 5-point scale. The two scores were then combined to form a measure of subjective sensory ability.[84]

Before calculating FAI, grip strength was adjusted for sex, and PEF was normalized for body size by dividing it by the square of the individual's height in meters.[84] Each of the four variables, gait speed, grip strength, PEF, and subjective sensory ability, was standardized individually based on the means and standard deviations at baseline to address measurement variance.[84] Grip strength and PEF were reverse-scored so that higher values indicated lower physical performance.[84] Then, the composite score was created by summing the four variables with equal weighting, and the resulting score was standardized to a mean of 50 and SD of 10.

3.3.2 Frailty Index

FI is a measure of biological aging that captures decline at the physiological and functional levels. It is calculated as the ratio of health deficits present to the total number of deficits considered, based on the Rockwood deficit accumulation model.[86] In this thesis, FI was created using a broad range of self-reported symptoms, clinical signs, chronic diseases, and functional impairments to comprehensively assess aging at the physiological and functional levels. Table 4 lists the specific items considered in the FI calculation.

The total number of health deficits considered in the FI calculation was 42 for GENDER and SATSA and 41 for OCTO-Twin. To be included in the FI, the health deficits had at least 1% prevalence within each cohort.[133] In previous work, data imputation was performed for participants with 20% or fewer missing items to limit missingness to a maximum of 10%.[134] Participants with more than 20% missing items were excluded from the analyses.[134] In **Study II**, self-reported T2D was removed from the FI calculation since the same item was used to define metabolic health.

Table 4: List of possible items included in the FI calculation across studies

Type	General health and chronic conditions	Physical, functional, and sensory limitations	Mental well-being and health	Activities of daily living
Items	• General health status • Limited from doing things one would normally like to do due to health status • Anemia • Asthma • Persistent cough • Chronic bronchitis or emphysema • Allergies/allergic manifestations • Eczema • Herpes • Cataracts • Glaucoma • Arthritis • Rheumatoid arthritis • Osteoporosis • Sciatica • Gout • Gall bladder issues • Gastric ulcer • Liver disease • Kidney disease • Goiter or other gland problems • Type II Diabetes • Heart attack • Heart failure • Hypertension • Vascular spasm in leg • Circulation problems in arms and legs • Migraine • Stroke • Epilepsy • Cerebral hemorrhage or blood clot in brain • Cancer or leukemia	• Hip joint impairment • Neck pain • Shoulder pain • Speech impairment • Picking something up from the floor • Handling small things with your fingers • Showering and bathing • Getting in and out of bed • Dressing and undressing • Self-grooming • Walking • Trouble getting to the toilet in time • Traveling further distances • Housework • Preparing meals • Hearing acuity • Vision acuity	• Feeling lonely the past week • Feeling depressed the past week • Feeling happy the past week • Feeling tired the past week • Insomnia • Psychological problems	• Managing medications • Managing money • Using the telephone • Grocery shopping • Keeping body fit

3.3.3 Epigenetic Age Acceleration

Epigenetic age was derived using DNA methylation data from the 2016 VBS study within HRS, where DNA methylation levels were measured with the Infinium Methylation EPIC BeadChip (Illumina Inc., San Diego, CA, USA).[132] This assay measures DNA methylation at over 850,000 CpG sites across the genome, which are associated with aging and other health-related phenotypes.[132] HRS computed multiple epigenetic clocks based on these data, each designed to estimate biological aging through CpG methylation patterns predictive of chronological age, health outcomes, and mortality risk.[132] This thesis included five of the epigenetic clocks:

1. **Horvath-I**: One of the first multi-tissue epigenetic clocks, Horvath-I estimates DNA methylation age using 353 CpG sites across 51 different tissue types.[89] The clock was trained on 8,000 samples using data from multiple Illumina methylation array datasets, and it showed a high correlation with chronological age (r = 0.96-0.97).[132]
2. **Hannum**: The Hannum clock focuses on age prediction in blood using 71 CpG sites selected from the Illumina 450,000 array.[91] The clock was developed from methylation data of 656 individuals, ranging from 19 to 101 years old, and was one of the first blood-based clocks to correlate DNA methylation changes with aging.[91] It strongly correlated with chronological age (r = 0.96).
3. **Horvath-II**: An extension of the first Horvath clock, Horvath-I, Horvath-II was developed to enhance age prediction, specifically in skin, fibroblasts, and blood. It utilizes 391 CpG sites, including shared CpGs with the original Horvath and Hannum clocks.[95] Horvath-II shows robust correlations with age in multiple tissues and is less affected by variations in blood cell types than its predecessor.[95]
4. **PhenoAge**: This clock integrates 513 CpG sites associated with chronological age and a range of clinical biomarkers (e.g., albumin, glucose, and C-reactive protein), enabling the prediction of mortality and disease risk beyond chronological age.[92] PhenoAge was strongly associated with health outcomes such as mortality, health span, and physical function.[92]
5. **GrimAge**: The GrimAge incorporates 1,030 CpG sites linked to smoking history and protein biomarkers of physiological health (e.g., CRP, GDF-15, and PAI-1).[93]

This model was trained to predict lifespan and age-related diseases and has been shown to have substantial predictive value for mortality and morbidity risks.[93]

6. **DunedinPACE**: Unlike traditional epigenetic clocks, DunedinPACE measures the rate of biological aging rather than cumulative age. It estimates the average rate of physiological changes across multiple systems, such as cardiovascular, metabolic, and immune functions, using 19 biomarkers of aging.[94] This pace-of-aging metric was derived using elastic-net regression models trained on longitudinal biomarker data taken from the Dunedin Study at ages 26, 32, and 38 .[94] The resulting estimates, expressed as "years per chronological year (year/chronological year)," quantify the pace of biological aging relative to the actual chronological age, with higher values indicating faster aging.

Epigenetic Age Acceleration (EAA) was calculated by adjusting each clock's estimated biological age for chronological age (except for DunedinPACE, which measures the pace of aging). This adjustment was done by regressing chronological age from each clock's estimate, with the resulting residuals representing how much an individual's biological age deviates from their chronological age. In the case of GrimAge, sex was also included in the regression model due to its role in the clock's construction.[135] A positive EAA indicates accelerated aging, where biological age exceeds chronological age, while a negative EAA suggests decelerated aging. The resulting EAA measures were labeled HorvathAgeAcc-I, HorvathAgeAcc-II, HannumAgeAcc, PhenoAgeAcc, and GrimAgeAcc.

3.3.4 Survival Outcomes

All-cause mortality, a clear and definitive endpoint, was used to capture overall mortality risk and assess population health in **Study I** and **Study III**. For the STR cohorts, survival data such as vital status and dates of death (when available) were obtained through linkages with the Swedish Tax Agency, using the unique personal identification numbers assigned to Swedish residents.

In the HRS, respondents' vital status was monitored through interviews with the respondents, their spouses or partners, or other knowledgeable individuals. When a

respondent passed away, these contacts typically reported the date of death. If this information was not directly available, the date of death was imputed based on the last known date the respondent was confirmed alive and the subsequent confirmation of their death.

3.3.5 Other Variables

Demographic variables, such as chronological age and sex (biological sex at birth), along with lifestyle factors like educational attainment and smoking status, were included in all studies within this thesis. Sex was dichotomized into male and female across all studies.

In the STR, education was categorized into seven years or less of education and more than seven years, which correspond to basic and more than basic education in Sweden. In the HRS, education was divided into two categories: those with a college or university education and above, versus those with less than a college or university education.

Smoking status was also categorized into two groups in STR: ever smokers and never smokers, and three groups in HRS: ever smokers, never smokers, and current smokers. A variable on the data source, categorized based on the sub-studies included in the research, was included in **Study I, II,** and **IV** to adjust for between-study heterogeneity. This variable had three categories: Gender, OCTO-Twin, and SATSA (coded numerically in **Study IV** for DCSM). In **Study III,** using data from HRS, models were adjusted for ethnicity and race based on self-reports and grouped into three categories: White/Caucasian, Black/African American, and other.

History of CVD was included as a variable in the models for **Study I**. It was defined based on self-reported conditions such as angina pectoris, myocardial infarction, hypertension, angina, thrombosis in the legs, ischemic stroke, or hemorrhagic stroke. Additionally, the year of exposure measurement in 10-year intervals (1985 to before 1995, 1995 to before 2005, 2005 to before 2015) was included in sensitivity analysis in Study **II** to assess the period effects of changing trends in prevalence and incidence of obesity and metabolic unhealthy status over time.

3.4 General Overview of Statistical Approaches

This thesis employs different regression-based models to investigate the relationships between BMI, metabolic health, biological aging, and all-cause mortality. Regression models are a cornerstone of statistical analysis in epidemiological research, allowing us to quantify the association between an exposure (e.g., BMI) and an outcome (e.g., survival time) while controlling for potential confounding factors.[136] These models are flexible and can accommodate different types of outcome variables (e.g., continuous, binary, time-to-event) and predictor variables.[136]

In this thesis, linear and nonlinear regression techniques are employed to capture the often complex and sometimes nonlinear associations between exposures and outcomes. Other regression methods, including mixed-effects linear models, Cox proportional hazards models, parametric survival models, and mediation analysis, are applied to address specific research questions. Additionally, the thesis utilizes the dual change score model (DCSM), a form of structural equation modeling, to investigate bidirectional relationships over time, offering deeper insights into the dynamic interactions between BMI and biological aging. All parameter estimates from these analyses were reported with 95% confidence intervals (CI).

3.4.1 Linear Regression

Linear regression is a statistical method used to examine the association between a continuous dependent variable and one or more independent variables. The goal is to model how the mean of a dependent variable (Y) depends linearly on the values of independent variables ($X_1, \ldots, X_p$). This relationship is expressed as:

$$E(Y|X) = \beta_0 + \beta_1 X_1 + \ldots + \beta_p X_p + \epsilon$$

where $E(Y|X)$ denotes the conditional mean of *Y* given the independent variables $X_1,\ldots,X_p$, β_0 the intercept, and $\beta_1, \ldots \beta_p$ denote the regression coefficients represent the expected change in Y for a unit change in the corresponding independent variable. ϵ is the error term accounting for the deviation of the observed values from the predicted values. Key assumptions must be met to yield reliable results from linear regression: linearity between dependent and independent variables, independence between error terms

for separate observations, homoscedasticity (equal variance in residuals across values of *X*), normal distribution of the error terms, and no multicollinearity between independent variables.

3.4.2 Mixed-effects Models

Mixed-effects models are a class of regression models useful when data have a longitudinal or clustered structure, specifically when observations are grouped into units, such as twins within a twin pair, individuals within healthcare facilities, or repeated observations within individuals.[137] In cases where data are structured longitudinally, in clusters or in a nested fashion, the assumption of independent observations in linear regression may be violated. Mixed-effects models can then be applied in such data structures to recognize this dependency by incorporating both fixed and random effects.[137] Fixed effects are the overall linear effects of an independent variable on an outcome, which are constant across all individuals or groups.

On the other hand, random effects capture variation that occurs due to the grouping or clustering of the data. Random effects can be included to let the intercepts and/or slope parameters vary randomly across groups or individuals, capturing variability that cannot be explained only by fixed effects alone and accounting for correlations between observations. The equation for a linear mixed-effects model with random intercepts and random slopes can be expressed as:

$$Y_{ij} = \beta_0 + \beta_1 X_{ij} + \mu_{0j} + \mu_{1j} X_{ij} + \epsilon_{ij}$$

where Y_{ij} denotes the observed dependent variable for individual i in group j, β_0 the fixed intercept (mean intercept across all groups), $\beta_1 X_{ij}$ the fixed effects of observed independent variable X_{ij} on the observed dependent variable Y_{ij} that is consistent across all groups, μ_{0j} the random intercept for group j, indicating how much the intercept for group j deviates from the fixed intercept β_0, $\mu_{1j} X_{ij}$ denote the random slope for group j, representing the deviation of group j's slope from the fixed slope β_1, and ϵ_{ij} represents the measurement error for individual i in group j.[138]

The assumptions in linear models apply to mixed-effects models, except that in mixed-effects models, there can be non-independence in observations. Additional assumptions include the normality of random effects and independence between random effects and error terms.

3.4.3 Cox Proportional Hazards Models

The Cox proportional hazards regression model is a statistical method used to describe the relationship between the time to an event (such as death or disease onset) and one or more predictor variables in the presence of censoring.[139] It is one of the most common models used in time-event-analysis in medical research.[139] The model builds on the hazard (or risk) of the event occurring at any given time, conditional on the covariates (predictors). An essential feature of the Cox model is its semi-parametric nature — it does not require specification of the baseline hazard function (which describes the hazard when all predictors are zero), providing flexibility to accommodate different hazard shapes. Only the effects of the covariates on the baseline hazard, the hazard ratios, are directly estimated. The model is written as:

$$h(t|X) = h_0(t)\exp(\beta_1 X_1 + \ldots + \beta_p X_p)$$

where $h(t|X)$ denotes the hazard at time t given predictors $X_1,\ldots,X_p$, $h_0(t)$ the baseline hazard function, and $\beta_1,\ldots,\beta_p$ represent the coefficients of the independent variables on a log hazard ratio scale.[139] A central assumption of the Cox model is that the ratio of hazards between the groups is constant over time.[139] The proportional hazards assumption can be tested using the scaled Schoenfeld residuals test**,** which assesses whether there is a time-dependent relationship between the Schoenfeld residuals and time. A random scatter of residuals over time indicates that the hazard ratios are constant, thus supporting the proportional hazards assumption.

3.4.4 Parametric Proportional Hazards Models

Parametric proportional hazards (PPH) models are a type of time-to-event analysis where the time-to-event (or survival time) follows a specific continuous parametric distribution, such as Exponential, Weibull, or Gompertz.[139] Like Cox models, PPH models assume the proportionality of hazards. However, unlike Cox models, the baseline hazard

function in PPH is fully specified and changes based on the selected parametric distribution. This explicit specification allows the model to fit the data more precisely. Still, it requires selecting a distribution that represents the underlying hazard function to avoid bias in the estimates.[139] The key difference between PPH and Cox models lies in the specification of the baseline hazard function. While Cox models leave the baseline hazard unspecified, PPH models define it explicitly according to the selected distribution (e.g., Exponential, Weibull, or Gompertz). The PPH model is expressed similarly to Cox models but with a specific form for $h_0(t)$, the baseline hazard. In the Gompertz PPH model, for example, the baseline hazard is replaced with $\lambda exp(\gamma t)$, where is the λ baseline hazard rate and γ is the shape parameter that controls the increase in hazard over time. The Gompertz PPH model is written as:

$$h(t|X) = \lambda exp(\gamma t)\exp(\beta_1 X_1 + \ldots + \beta_p X_p)$$

where $h(t|X)$ denotes the hazard at time t given predictors $X_1,\ldots,X_p$, and $\beta_1,\ldots,\beta_p$ represent the coefficients of the predictors on a log hazard ratio scale.[140, 141] In this equation $\lambda exp(\gamma t)$ replaces $h_0(t)$ as the baseline hazard function, while t is still the time variable.

One of the advantages of PPH models is that, in addition to estimating hazard ratios, they allow for straightforward calculation of survival probabilities, hazard rates, and survival times based on the assumed distribution.[139] The survival function for the Gompertz PPH model is derived from the cumulative hazard and is expressed as:

$$S(t|X) = \exp\left(-\frac{\lambda}{\gamma}(exp(\gamma t) - 1)\, exp\big(\beta_1 X_1 + \ldots + \beta_p X_p\big)\right)$$

Where $S(t|X)$ represents the survival probability at time t, given the covariates $X_1,\ldots,X_p$.[140, 141]

3.4.5 Mediation Analysis

Mediation analyses aim to disentangle pathways, mechanisms, and mediators through which a cause influences an outcome.[142] There are different approaches to mediation analysis. Traditional techniques, such as the difference method and the product-of-

coefficients method[142] will not be discussed here; I will focus on mediation analysis within the counterfactual, or potential outcomes framework, as described by Imai et al.[143] This is the approach adopted in **Study III**. This framework allows us to estimate mediation effects by comparing potential outcomes under different conditions of the exposure variable and the mediator.

Mediation analysis using the counterfactual framework follows the general structure of traditional mediation models, in which two models are specified: one for the mediator as a function of the exposure (mediator model) and another for the outcome as a function of both the exposure and the mediator (outcomes model). However, instead of expressing direct and indirect (mediated) effects through regression coefficients, the counterfactual approach estimates these effects as differences in outcomes between hypothetical scenarios — comparing an exposed group to an unexposed group.[143]

In line with the terminology used by Imai et al.,[143] the exposed group is referred to as the treatment group (treated), and the unexposed group is the control group (not treated). To estimate mediated or indirect effects, the treatment status is held constant while adjusting the mediator to its value under either the treated or untreated condition, with all covariates left at their observed values. Let $M_i(T_i)$ represent the potential value of mediator for individual i under treatment T_i, and $Y_i(T_i, M_i(T_i))$ denote the potential outcome for the individual i, where the outcome is a function of both the treatment and the mediator. The indirect effect can then be defined as the difference in potential outcomes when the mediator changes between the treatment (being treated, $T = 1$) and control (not treated, $T = 0$) conditions while holding the treatment status constant:[143]

$$\delta_i(T) = Y_i\big(T, M_i(1)\big) - Y_i\big(T, M_i(0)\big)$$

where $\delta_i(T)$ represents the mediated or indirect effect for individual i. The direct effect is defined as the change in the potential outcomes caused by the treatment, with the mediator held constant at a fixed level:

$$\zeta_i(T) = Y_i\big(1, M_i(T)\big) - Y_i\big(0, M_i(T)\big)$$

where $\zeta_i(T)$ represents the direct effects for individual i.

The average causal mediation effect (ACME) is the average causal mediation effects of a population, denoted as $\delta(T)$ and written as:

$$\delta(T) = E\left[Y_i\left(T, M_i(1)\right) - Y_i\left(T, M_i(0)\right)\right]$$

The average direct effect (ADE) is the average direct effects of a population denoted as $\zeta(T)$ and written as:

$$\zeta(T) = E\left[Y_i\left(1, M_i(T)\right) - Y_i\left(0, M_i(T)\right)\right]$$

A crucial aspect of mediation analysis is ensuring that indirect and direct effects can be interpreted causally, which requires meeting four key assumptions.[142] One is that there is no unmeasured confounding between treatment(exposure) and outcome.[142] Two, that there is no unmeasured confounding between mediator and outcome. Three, there is no unmeasured confounding between treatment and mediator.[142] Four, there is no confounder of the mediator outcome that is affected by the treatment (exposure).[142]

3.4.6 Restricted Cubic Splines

Regression models typically assume a linear relationship between the dependent (outcome) variable and independent variables. However, not all associations follow a linear pattern. One can use transformations such as the polynomial functions, logarithms, or splines to capture the true shape of non-linear relationships.[144] This section focuses on the use of splines, specifically restricted cubic splines (RCS), as applied in **Studies II** and **III**.

Restricted cubic splines, also known as natural splines, are piecewise cubic polynomials used to model the relationship between dependent and independent variables.[144] They offer flexibility to fit complex, non-linear patterns while ensuring smooth transitions between polynomial segments.[144] The "restricted" aspect refers to the constraint RCS imposes at the tails, where the spline is forced to become linear, ensuring stable and interpretable fits.[144] In contrast, unrestricted cubic splines do not have this constraint, which can lead to unstable behavior at the extremes.

When specifying RCS, the number and location of knots must be chosen.[145] Knots are points where the cubic polynomials meet, and a separate cubic function is fitted between each pair of knots. For example, when using three knots, they are typically placed at the 25th, 50th, and 75th percentiles of the independent variable. This results in one cubic polynomial fitted between the 25th and 50th percentiles and another between the 50th and 75th percentiles. Linear functions are applied to the data below the 25th percentile and above the 75th percentile.

3.4.7 Dual Change Score Models

DCSMs are a specialized form of structural equation modeling used to explore how changes in one variable may influence changes in another while accounting for linear and nonlinear trajectories over time.[146] These models are valuable for investigating bidirectional relationships, where both variables can potentially impact each other's rate of change. DCSMs decompose change into two key components: constant change and proportional change.[146] The constant change reflects the expected steady trajectory over time. In contrast, proportional change accounts for any nonlinear shifts by linking the rate of change at a given point to the variable's value at the previous time point.

3.4.7.1 Univariate DCSM

Before examining bidirectional association, univariate DCSMs are applied to model each variable's baseline level (intercept) and trajectory separately. The proportional change parameter (β) captures whether the rate of change is influenced by the variable's previous level.[147] Taking BMI as an example, the change in BMI from one time to the next (ΔBMI_t) can be expressed as:

$$\Delta BMI_t = \alpha \left(BMI_{slope}\right) + \beta \left(BMI_{t-1}\right)$$

Here, BMI_{slope} represents the constant linear rate of change in BMI, while β reflects any additional non-linear change that depends on the value of the BMI at the previous time point (BMI_{t-1}). The trajectories produced from univariate models with a single β and α set to one are typically exponential in shape, showing increasing and decreasing trends.[147]

3.4.7.2 Bivariate DCSM

Once the univariate trajectories are modeled, a bivariate DCSM is employed to assess how changes in the two variables — such as BMI and a biological age measure (BA) — are interrelated over time. Bivariate DCSMs estimate coupling parameters (γ), which quantify how changes in one variable (e.g., ΔBMI) are associated with changes in the other (e.g., ΔBA).[147] Accounting for such coupling effects from BMI, change in BMI from one-time point to the next can be expressed as:

$$\Delta BMI_t = \alpha\left(BMI_{slope}\right) + \beta\left(BMI_{t-1}\right) + \gamma_{BA\rightarrow\Delta BMI}\left(BA_{t-1}\right)$$

This equation includes the same parameters as the univariate model above and, in addition, a coupling parameter, $\gamma_{BA\rightarrow\Delta BMI}$ which captures the effect of BA from the previous level on the rate of change of BMI.[147] Similarly, changes in BA (ΔBA_t) can be modeled as:

$$\Delta BA_t = \alpha\left(BA_{slope}\right) + \beta\left(BA_{t-1}\right) + \gamma_{BMI\rightarrow\Delta BA}\left(BA_{t-1}\right)$$

Here, the same logic applies: ΔBA_t denotes the change in BA at time t and $\gamma_{BMI\rightarrow\Delta BA}$ reflects the influence of prior BMI levels on the change in BA.

Coupling between two variables, such as BMI and BA, can manifest in four distinct ways:

1. Bi-directional coupling: Changes in BMI influence changes in BA, and at the same time, changes in BA also influence changes in BMI ($\gamma_{BMI\rightarrow\Delta BA} \neq 0;\ \gamma_{BA\rightarrow\Delta BMI} \neq 0$)
2. Unidirectional coupling (BMI→BA): Changes in BMI affect the rate of change in BA, but BA does not affect changes in BMI ($\gamma_{BMI\rightarrow\Delta BA} \neq 0;\ \gamma_{BA\rightarrow\Delta BMI} = 0$)
3. Unidirectional coupling (BA→BMI): Changes in BA affect the rate of change in BMI, but BMI does not affect changes in BA ($\gamma_{BMI\rightarrow\Delta BA} = 0;\ \gamma_{BA\rightarrow\Delta BMI} \neq 0$)
4. No coupling: Changes in BMI and BA are independent of each other, implying no predictive relationship between the two variables over time ($\gamma_{BMI\rightarrow\Delta BA} = 0;\ \gamma_{BA\rightarrow\Delta BMI} = 0$)

To identify the best-fitting model, we may compare different coupling scenarios using likelihood ratio tests for nested models (LRTs), log-likelihood ratio (-2LL), and Akaike Information Criterion (AIC). The model that provides the most accurate

representation of the data while balancing complexity is selected, and this best-fitting model indicates the direction of the coupling between the variables.

3.4.7.3 Flexibility with Breakpoints

Breakpoints can be introduced into the model to accommodate changes in the strength or direction of associations at different life stages. These breakpoints allow the proportional change parameter (β) and coupling parameter (γ) to vary at specific ages, providing flexibility in modeling how the relationships evolve over time. Models with different numbers of breakpoints were compared using criteria such as AIC and LRT to determine the best-fitting model.

3.4.7.4 Adjusting for Covariates

DCSMs also allow for the inclusion of covariates to adjust for potential confounding factors, such as sex or smoking status. Additionally, the variance and covariance of the intercepts and slopes are estimated to capture individual differences in baseline levels and rates of change for both variables. Figure 4 illustrates an example of how dual change score models can represent the dynamic relationship between BMI and biological age over time, with adjustments for individual-level characteristics like sex and smoking, as a path diagram.

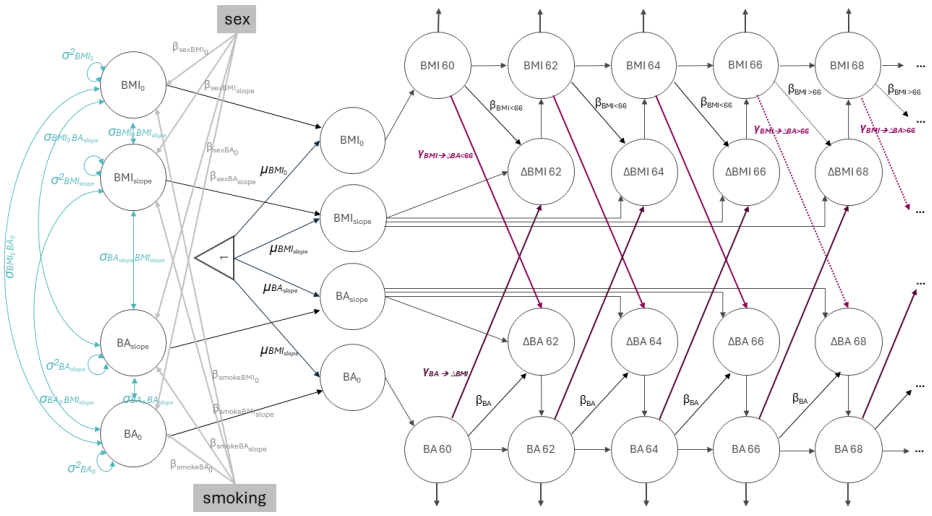

Figure 4: Sample path diagram of a bivariate dual change score model of BMI and BA

This sample diagram illustrates the relationship between BMI and BA across several time points, specifically from ages 60 to 68. In practice, the full model may extend to account for changes over a broader age

- **Time Points:** BMI 60–68 and BA 60–BA 68 represent BMI and BA levels at ages 60-68, with each age bin covering approximately two years (e.g., 60-61.9, 62-63.9, etc.)
- **Intercepts and Slopes:** BMI_0 and BA_0 denote the baseline levels at age 60. BMI_{slope}, BA_{slope} denote the constant linear change for BMI and BA over time
- **Mean Levels, variances, and covariances**: μBMI_0, μBMI_{slope}, μBA_0, and μBA_{slope} represent the mean levels for intercepts and linear slopes of BMI and BA, ($\sigma^2 BMI_0$, $\sigma^2 BMI_{slope}$, $\sigma^2 BA_0$, $\sigma^2 BA_{slope}$) corresponding variances. Covariance is denoted by σ, so $\sigma BMI_0 BMIslope$, $\sigma BMI_0 BA_{slope}$, $\sigma BMI_0 BA_0$,$\sigma BMI_0 BA_{slope}$, etc. represent the covariance between BMI and BA intercepts and slopes.
- **Changes in BMI and BA Over Time:** ΔBMI 62 to ΔBMI 68 and ΔBA 62 to ΔBA 68 represent changes between time points. These are linked by slopes(BMI_{slope}, BA_{slope})representing linear effects, and proportional parameters(β_{BMI}, β_{BA}) capturing non-linear effects
- **Coupling Parameters:** $\gamma_{BMI \rightarrow \Delta BA}$ links BMI at one time point to BA change, and $\gamma_{BA \rightarrow \Delta BMI}$ links BA to BMI change.
- **Covariate Adjustments:** The model accounts for sex and smoking through parameters like β_{sexBMI_0}, $\beta_{smokeBMIslope}$, β_{sexBA_0} and $\beta_{smokeBAslope}$
- **Breakpoints in proportional change and coupling:** The diagram illustrates, for example, a breakpoint at age 66. Separate proportional change parameters are estimated for BMI before ($\beta_{BMI<66}$) and after age 66 ($\beta_{BMI>66}$). Likewise, coupling parameters for BMI's effect on BA are split: $\gamma_{BMI \rightarrow \Delta BA<66}$ (before age 66) and $\gamma_{BMI \rightarrow \Delta BA>66}$ (after age 66).

3.5 Ethical Considerations

This thesis draws on data from sub-studies within the STR and the HRS. As the analyses are conducted in Sweden, they are governed by Swedish ethical regulations, specifically Law 2003:460,[148] which ensures the protection of human rights and dignity in research. Ethical permits for the studies in this thesis were obtained from the Swedish Ethical Authority. Table 5 shows the ethical approvals for each study included in this thesis.

Table 5: Ethical approvals for each study

Study	Ethical approval numbers
Study I	2015/1729 – 35/5
Study II	2015/1729 – 35/5, 2022-06634-0
Study III	2015/1729 – 35/5, 2019-02588, 2022-06634-01, 2024-03706-0
Study IV	2015/1729 – 35/5, 2022-06634-01, 2024-03706-0

This thesis relies on previously collected data, which reduces ethical risks since there was no direct contact with participants and no new data collection. Informed consent was obtained from all participants in the original studies, both STR and HRS.[120, 149] Despite the lack of new participant involvement, using sensitive personal and health data from these studies still presents potential risks, mainly related to confidentiality and re-identification. The data include sociodemographic information, medical histories, physical and cognitive assessments, and biological samples. STR and HRS have established rigorous protocols for de-identification and data security to protect participants' privacy, ensuring access is limited to approved researchers through secure, controlled systems.[120, 149]

In the HRS, data are de-identified, and personal identifiers such as names and addresses are stored separately on secure servers, accessible only to authorized personnel at the Survey Research Center.[149] The HRS data undergo a three-stage iterative process to ensure confidentiality before being made available to researchers.[149] Public data are distributed through a secure, password-protected

website, while more sensitive data are released after additional application processes.[149] HRS holds a Federal Certificate of Confidentiality, which protects participants from being identified in legal proceedings.[149]

STR data are pseudonymized.[120] The keycodes linking data to personal identifiers are securely stored by principal investigators according to Swedish regulations, and the datasets provided to researchers contain no direct personal identifiers.[120, 150]

All datasets used in this thesis are securely stored on servers at Karolinska Institutet, with access restricted to authorized researchers only. Remote access to the data is enabled through a secure virtual desktop infrastructure that is password-protected and requires multi-stage authentication within the network at Karolinska Institutet. The data has not been and will not be stored on personal devices or third-party cloud services, and data sharing through insecure methods like email or messaging platforms is strictly prohibited.

While randomized controlled trials (RCTs) are the gold standard for establishing causality in medical research, conducting RCTs to study disease risk factors would often be unethical. Leveraging epidemiological data, such as that from STR and HRS, allows for the exploration of long-term trends and associations across large populations without exposing participants to new risks. This thesis benefits from the extensive longitudinal data on aging, metabolic health, and BMI trajectories provided by these data sources. The research reduces ethical risks by using pre-existing data while yielding valuable insights into public health.

This thesis's use of inclusive and respectful language is a key ethical consideration. While terms like "obesity" are scientific, the focus is to avoid stigmatizing language, such as referring to "obese individuals" or "obese people."[151] Instead, neutral and person-first terminology like "individuals with high BMI" or "people with high BMI" were used as much as possible to emphasize the person rather than defining them by their condition. Similarly, terms like "elderly" have been avoided in favor of more neutral phrases like "older adults" or "older people," aligning with the guidelines provided by the American Medical Association to reduce ageism in research language.[152] These efforts ensure that

the language used throughout the thesis is respectful and avoids perpetuating stereotypes or stigmatization.

Like many others, this thesis has only been made possible by the voluntary participation of individuals in both the STR and HRS. While the anonymous participants may not directly benefit from their involvement, their contributions are essential in improving our understanding of aging, disease prevention, and public health, benefitting current and future generations.

3.6 Study I to IV — The Big Picture

In this section, I will delve into each study's specific methods, results, and methodological challenges. Figure 5 provides an overview of all four studies, offering a visual snapshot of their key themes and contributions.

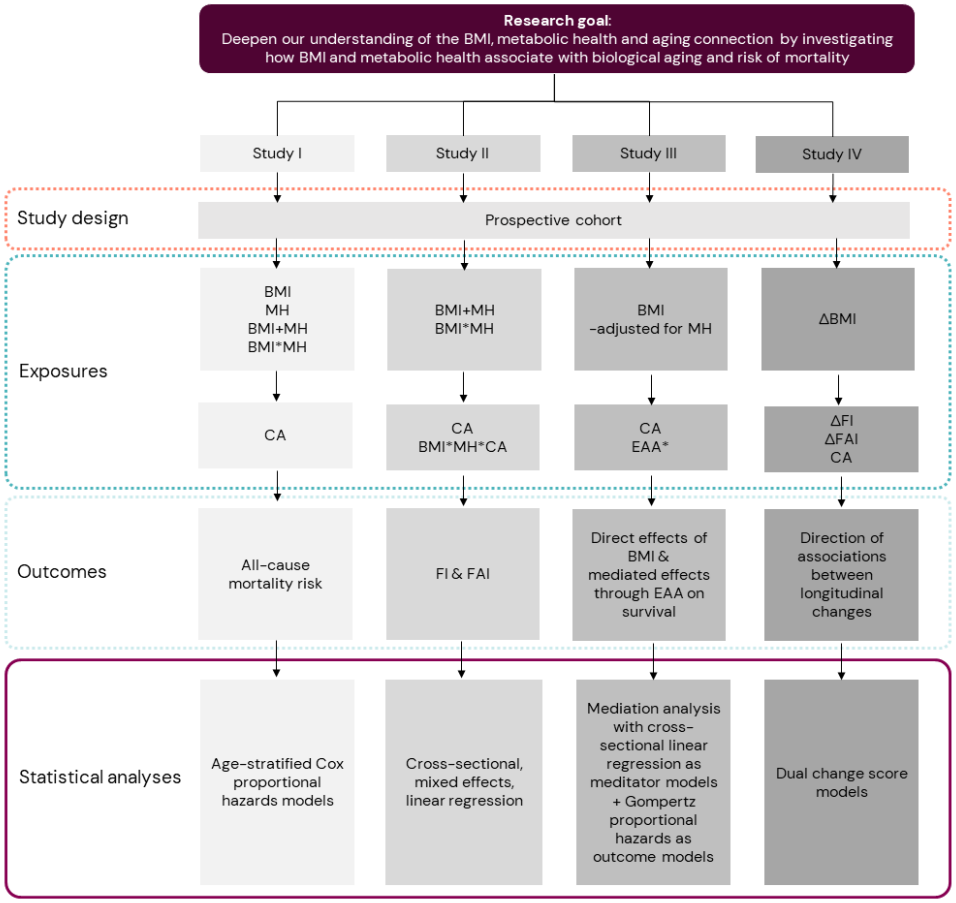

Figure 5: Overview of all four studies

*indicates role as a mediator

Abbreviations: BMI – body mass index, CA – chronological age, EAA – epigenetic age acceleration, FAI – functional aging index, FI – frailty index, MH – metabolic health status

4 Results

4.1 Study I — Mortality Risk Linked to Metabolic Health, Regardless of BMI

This study had two main objectives. First, we aimed to understand how BMI and metabolic health independently and jointly associate with the risk of all-cause mortality. Second, we explored whether these associations differed depending on whether BMI and metabolic health were assessed in midlife or late life.

4.1.1 How We Got Here: Methods Overview

This study pooled data from GENDER, OCTO-Twin, SATSA, and TwinGene from 1958 to 2008, with mortality data linked through December 31st, 2020. This study used BMI as a categorical variable (normal weight, overweight, and obesity). Underweight was excluded due to the low sample size. We examined the mortality risk associated with an interaction between BMI categories and metabolic health, resulting in metabolic-BMI phenotypes (see Figure 2): MHN — reference group, MUN, MHOw, MUOw, MHO, and MUO.

Cox proportional hazard regression models were applied, with attained age as an underlying timescale to assess the associations of BMI, metabolic health, and metabolic-BMI phenotypes with all-cause mortality, adjusted for sex, education, smoking, and CVD history. The analyses were stratified into midlife (measures taken aged ≤ 65) and late life (measures taken aged > 65) to examine age-specific effects.

The proportional hazards assumption was tested with Schoenfeld residuals for each independent variable. CVD in midlife and smoking in late life, which did not meet proportionality requirements, were incorporated in the models as time-varying covariates.

4.1.1.1 Sensitivity analyses

Extensive sensitivity analyses were conducted with metabolic-BMI phenotypes as the primary exposure. Firstly, since this was the first study where we used metabolic health as a variable within this dataset, and there is no agreement on the definition of metabolic health, we tested different ways of operationalizing it, including:

- adding CVD history as a component in the metabolic health definition;

- excluding self-reported data, such as use of diabetes medications, lipid-lowering drugs, and diagnoses of diabetes;
- defining metabolic health as the absence of any metabolic abnormalities;
- adding WC as an additional criterion for determining metabolic health, with cut-off points set at 80 cm for females and 94 cm for males to indicate metabolic abnormalities

Secondly, we further adjusted the models with BMI history as a categorical variable to assess bias and reverse causality. BMI history was the highest BMI recorded in ages 45 to 65, at least five years before baseline. Thirdly, the entire sample was stratified by sex to examine sex differences. Lastly, we examined all the individual metabolic components within a single model.

4.1.2 What We Found

We analyzed 12,467 individuals, with a mean follow-up period of 13 years. A total of 6,252 individuals, with a mean age of 60 years, were followed up from midlife for an average of 14 years, while 6,215 individuals, with a mean age of 73 years, were followed up from late life for an average of 12 years.

Independently, obesity in both midlife and late life was associated with a 42% and 22% higher risk of mortality, respectively, compared to normal weight (see Table 6). Overweight in both midlife and late life, however, was not significantly associated with increased mortality risk. Being metabolically unhealthy in midlife and late life was associated with a 43% and 25% higher risk of mortality, respectively, compared to being metabolically healthy.

Jointly, the hazard ratios (HRs) for midlife and late-life obesity weakened to 15% and 25% higher mortality risk, respectively. The HRs for being metabolically unhealthy remained consistent. The HRs for midlife and late-life obesity and metabolic health were attenuated in independent and joint models when adjusted for CVD.

Table 6: Risk of all-cause mortality in relation to body mass index and metabolic health status, independently and jointly

Age Strata	Models	BMI independently		MHS independently		BMI and MHS jointly	
		Model 1 HR (95% CI)	Model 2 HR (95% CI)	Model 3 HR (95% CI)	Model 4 HR (95% CI)	Model 5 HR (95% CI)	Model 6 HR (95% CI)
	Adjustments	Sex, Educ, Smoke	+ CVD	Sex, Educ, Smoke	+ CVD	Sex, Educ, Smoke	+ CVD
≤65 years	BMI categories (*Reference: Normal weight*)						
	Overweight	1.02 (0.87 – 1.21)	0.99 (0.84 – 1.16)			0.96 (0.81 – 1.13)	0.94 (0.79 – 1.12)
	Obesity	**1.42 (1.16 - 1.75)**	**1.30 (1.06 – 1.60)**			**1.25 (1.00 – 1.55)**	1.19 (0.96 – 1.48)
	Metabolic health status (*Reference: Metabolically healthy status*)						
	MU status			**1.43 (1.23 – 1.66)**	**1.31 (1.12 – 1.53)**	**1.38 (1.18 – 1.62)**	**1.28 (1.09 – 1.51)**
>65 years	BMI categories (*Reference: Normal weight*)						
	Overweight	0.99 (0.93 – 1.07)	0.96 (0.89 – 1.03)			0.96 (0.89 – 1.03)	0.94 (0.87 – 1.01)
	Obesity	**1.22 (1.10 – 1.35)**	**1.15 (1.04 – 1.27)**			**1.15 (1.04 – 1.28)**	**1.11 (1.01 – 1.23)**
	Metabolic health status (*Reference: Metabolically healthy status*)						
	MU status			**1.25 (1.17 – 1.34)**	**1.18 (1.10 – 1.26)**	**1.25 (1.16 – 1.33)**	**1.18 (1.10 – 1.26)**

Hazard ratios with 95% confidence intervals from Cox regression models of all-cause mortality in relation to body mass index and metabolic health status jointly and independently. Models 1, 3, and 5 were adjusted for sex, education attainment, and smoking status. Models 2, 4, and 6 were adjusted for sex, education attainment, smoking status, and history of cardiovascular disease. Bold numbers indicate significance at the α=0.05 level.

Abbreviations: HR – hazard ratios, CI – confidence interval, Educ – education attainment, Smoke – smoking status, + CVD – additionally adjusted with history of cardiovascular disease, BMI – body mass index, MU – metabolically unhealthy

In the interaction models (see Figure 6), MUOw and MUO in midlife were associated with a 31% and 73% higher risk of mortality, respectively, compared to MHN. In contrast, neither MHOw nor MHO in midlife was associated with a higher mortality risk. In late life, being metabolically unhealthy was associated with higher mortality risk regardless of BMI: MUN, MUOw, and MUO were associated with 21%, 20%, and 43% higher mortality risk compared to MHN, respectively. However, MHO in late life was not significantly associated with mortality risk. Interestingly, late-life MHOw was associated with lower mortality risk in models adjusted for CVD.

4.1.2.1 What we found in sensitivity analyses

Firstly, we found that different ways of defining metabolic health changed the magnitude but not the direction of the associations.

When BMI history was added to the model, the mortality risks associated with the metabolic health-BMI phenotypes were attenuated and generally no longer statistically significant (see Figure 6). The BMI history in the midlife stratum was not significantly associated with an increased mortality risk. However, in the late-life sample, BMI history had little impact on the increased mortality risk in MUN. Additionally, reduced risk of mortality in late-life MHOw reached statistical significance. A history of overweight and obesity in late-life stratum, on the other hand, was associated with an 11% and 32% increased risk of mortality compared to a history of normal weight, respectively.

No notable sex differences were observed in the findings, although hazard ratios were generally higher in males than females, especially during midlife (see Figure 7). In addition, we found that applying different definitions of MHS influenced the magnitude of the effects but did not alter the overall pattern or conclusions.

When we included all the metabolic health components separately in a single model, the BMI category in midlife and late life was not associated with mortality risk (see Table 7). Among the metabolic parameters defining metabolic health, hyperglycemia had the strongest association with all-cause mortality, increasing risk by 78% in midlife and 52% in late life compared to normoglycemia. In late life, hypertriglyceridemia raised mortality risk by 9%, though it was not statistically significant in midlife. Hypertension, BMI, and low HDL-C were not associated with mortality at any age. CVD in midlife was associated with an almost ninefold increase in mortality risk, but with a time-varying effect, reducing HRs by 3% per year of survival. In late life, CVD was associated with a 33% higher mortality risk.

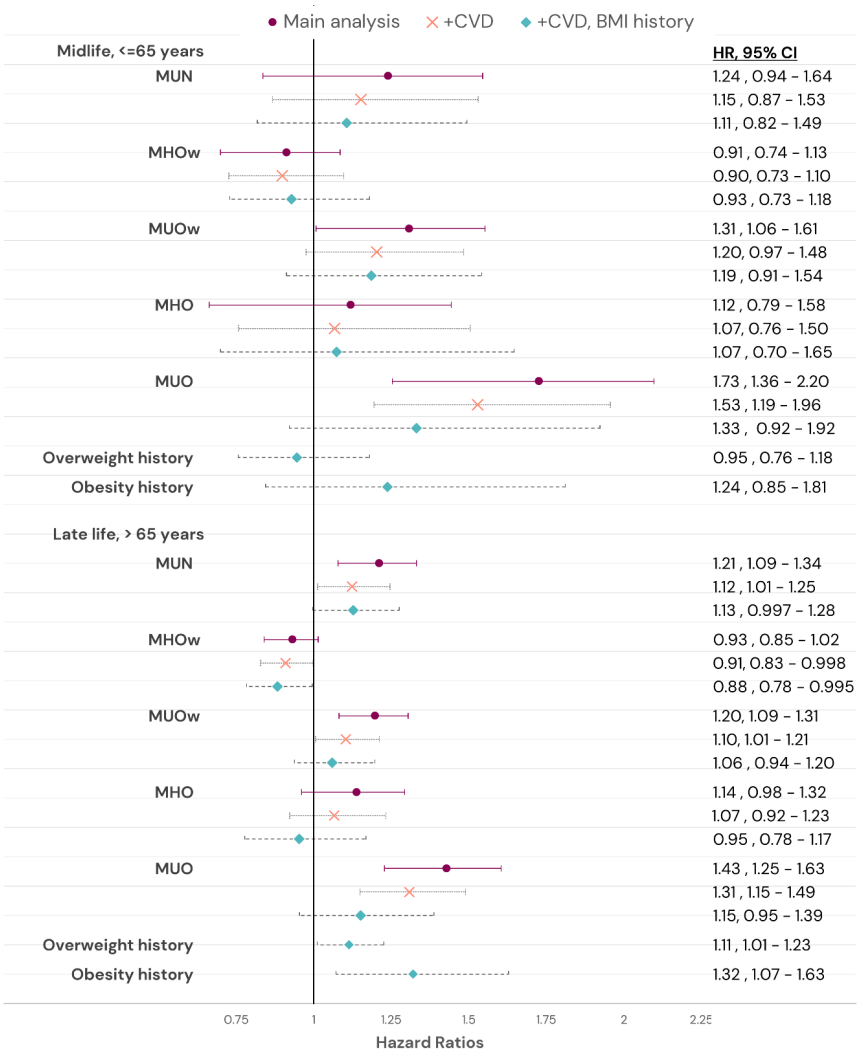

Figure 6: Hazard ratios and 95% confidence intervals of all-cause mortality

Main analysis refers to models adjusted for sex, education, and smoking, +CVD models are further adjusted further for history of CVD, + CVD, BMI History models were further adjusted for BMI history. The reference category for metabolic health-BMI phenotypes was metabolically healthy normal weight, for BMI history normal weight. Figure reproduced from Ler et al., BMC Public Health 2022.[153]

Abbreviations: CVD - history of cardiovascular disease, MUN – metabolically unhealthy normal weight, MHOw – metabolically healthy overweight, MUOw – metabolically unhealthy overweight, MHO – metabolically healthy obesity, MUO – metabolically unhealthy obesity, HR – hazard ratios, CI – confidence intervals

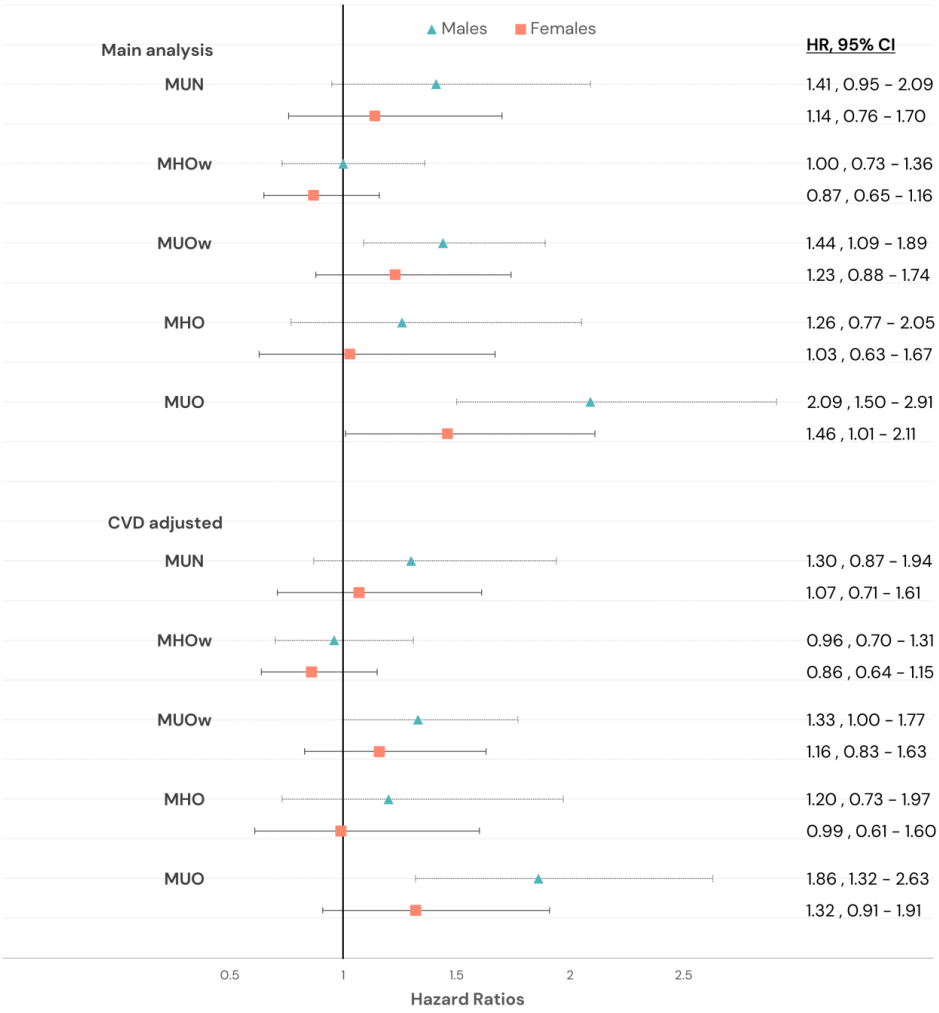

Figure 7: Hazard ratios and 95% confidence intervals of all-cause mortality by sex

Models adjusted for education and smoking. CVD adjusted models were further adjusted for history of CVD.

Abbreviations: MUN – metabolically unhealthy normal weight, MHOw – metabolically healthy overweight, MUOw – metabolically unhealthy overweight, MHO – metabolically healthy obesity, MUO – metabolically unhealthy obesity, HR – hazard ratios, CI – confidence intervals

Table 7: Association of individual metabolic components with all-cause mortality

Metabolic Components	Age Strata	
	≤65 years	>65 years
	HR (95% CI)	HR (95% CI)
Overweight (Reference = Normal weight)	0.95 (0.80 – 1.13)	0.93 (0.87 – 1.00)
Obesity (Reference = Normal weight)	1.13 (0.90 – 1.42)	1.10 (0.99 – 1.21)
Hypertension (Reference = Normatension)	1.00 (0.85 – 1.19)	0.93 (0.85 – 1.04)
Hyperglycemia (Reference = Normaglycemia)	**1.78 (1.43 – 2.20)**	**1.52 (1.38 – 1.67)**
Hypertriglyceridemia (Reference = Normaglyceridemia)	1.13 (0.92 – 1.36)	**1.09 (1.01 – 1.18)**
Low HDL-C (Reference = Normal HDL-C)	1.01 (0.83 – 1.22)	1.02 (0.94 – 1.11)
CVD History (Reference = No CVD History)	**8.94 (2.06 – 38.81)**	1.33 (1.25 – 1.43)
CVD History*Time (Reference = No CVD History)	**0.97 (0.95 – 0.99)**	

Hazard ratios with 95% confidence intervals of all-cause mortality in relation to BMI category and individual metabolic components, adjusted for sex, education, and smoking.

Abbreviations: CVD – cardiovascular disease, HR – hazard ratios, CI – confidence intervals

4.1.3 How We Tackled Challenges

As section *1.2.1.2* in Chapter 1 outlines, one of the major issues in this study is the potential for reverse causation. In the late-life group, the observed associations between MUN and MHOw with mortality risk could have been influenced by illness-induced weight loss, potentially exaggerating the risks for MUN and downplaying them

for MHOw. To mitigate this issue, we incorporated BMI history into our models, to account for BMI fluctuations that may be attributable to chronic illnesses. The attenuation but consistency of HRs after adjusting for BMI history suggests that the effects of reverse causation may have been minimized.

The classification of MHS presented another challenge. Using non-fasting glucose and lipid levels to classify hyperglycemia and hypertriglyceridemia may have introduced some misclassification. In the OCTO-Twin cohort, the lack of triglyceride data required reliance on self-reported use of lipid-lowering medications only, which may have overestimated hypertriglyceridemia. To account for concerns in the ascertainment of MHS, we performed comprehensive sensitivity analyses using alternative MHS definitions. Changes in MHS definitions yielded results with stable trends reinforcing the robustness of our findings.

In the midlife group, the 13.9-year mean follow-up increased the likelihood of capturing early deaths, particularly from CVD. The ninefold rise in CVD mortality risk in those with a history of CVD, which decreased over time as reflected by CVD history as a time-varying covariate, likely reflects survivor bias, where premature deaths disproportionately influence the observed associations (see Table 7).

Relying solely on baseline assessments for BMI and MHS limited our ability to capture changes over time, which can offer valuable insights into longitudinal effects, temporal dynamics, and disease progression or reversal. BMI is not the only variable subject to fluctuation; previous studies have demonstrated the transient nature of BMI-metabolic health phenotypes, showing how MUO can transition to MHO, or vice versa, over time.[81, 154, 155] Ongoing research within our group continues to explore the trajectories of these phenotypes to deepen our understanding of their dynamic changes.

Finally, BMI was categorized, and metabolic health was dichotomized as healthy or unhealthy based on specific thresholds. BMI-metabolic health categories were then created through the cross-categorization of these variables. While using categorical variables simplifies interpretation, facilitates comparisons across studies, and mirrors real-world clinical and policy contexts (especially for BMI), it may result in the loss of valuable information. Broad BMI categories can mask subtle, non-linear associations,

particularly within specific BMI ranges, and fail to capture individual variability. Similarly, simplifying metabolic health into binary categories may overlook intermediate metabolic profiles that could have important implications for health outcomes. These limitations highlight the need for more refined approaches, such as the continuous treatment of BMI, addressed in the following section of **Study II**.

4.2 Study II — Higher Biological Aging in Low BMI, High BMI, and Metabolically Unhealthy Status

Building on the findings from **Study I**, which highlighted the joint associations of midlife and late-life BMI and metabolic health with all-cause mortality, **Study II** shifts the focus to exploring how BMI and metabolic health from mid to late life influence functional and physiological aspects of biological aging. In this study, BMI was treated as a continuous variable while considering non-linearity, addressing the limitations of categorical BMI that can lead to a loss of critical information. This approach allows for a more nuanced analysis of the relationship between BMI, metabolic health, and aging, capturing non-linear effects and providing a comprehensive understanding of how BMI and metabolic health contribute to functional and physiological aging.

4.2.1 How We Got Here: Methods Overview

Study II utilized longitudinal data from the GENDER, OCTO-Twin, and SATSA cohorts, which included three, five, and ten waves of IPT. Linear mixed-effects models were employed to account for repeated measures within individuals over time and correlations within twin pairs. This approach allowed us to model the cross-sectional associations of BMI and metabolic health with FAI (FAI model) and FI (FI model) as separate outcomes while controlling for chronological age, sex, and education.

RCS were applied to account for potential non-linear associations between BMI, chronological age, and outcomes. We also tested three-way interactions between BMI, metabolic health, and chronological age to determine whether the joint association of BMI and metabolic health with FAI or FI was influenced by age.

Significant three-way interactions were found for FI but not for FAI. Visual inspection of predicted FI over chronological age showed that FI exhibited a mostly linear relationship within three age groups: under 65, 65 to under 85, and 85 and above. Based on this, we proceeded with age-stratified linear mixed-effects models for these intervals and reassessed the linearity and three-way interactions within each age group.

Sensitivity analyses were conducted in the full sample for FAI and each stratum for FI. We adjusted the models for the data source and the period during which the exposures

were collected, as well as included measure of central obesity (WHR) in the definition of metabolic health to assess their influence on the findings. Additionally, we investigated how individual metabolic components and BMI jointly associate with FAI and FI.

4.2.2 What We Found

The models included data from 1,691 individuals with 5,257 FAI measurements and 1,825 individuals with 6,051 FI measurements. The mean age for the FAI sample was 73.0 years (SD = 9.87), and for the FI sample, it was 73.6 years (SD = 9.93). The average BMI was 25.5 (SD = 4.01) for the FAI group and 25.6 (SD = 3.96) for the FI group. Approximately 23% of participants in both samples were classified as MU.

4.2.2.1 FAI Models

The results from the FAI models showed no significant three-way interactions between BMI, metabolic health, and chronological age, indicating that the association between BMI, metabolic health, and functional aging did not vary significantly across age groups [all p-value of interactions (p-interaction > 0.11), see Figure 8]. BMI was associated with FAI (Wald test p-value = 3.1E-7) in a nonlinear manner (Wald test p-value = 1.4E-4), where individuals with a BMI around 28.4 kg/m² had the lowest FAI. Lower and higher values of BMI were associated with higher FAI. Being metabolically unhealthy was associated with higher FAI across all ages [Beta-coefficient (β) = 1·46, 95% CI = 0·94—1·97, *p*-value = 2·9E-9].

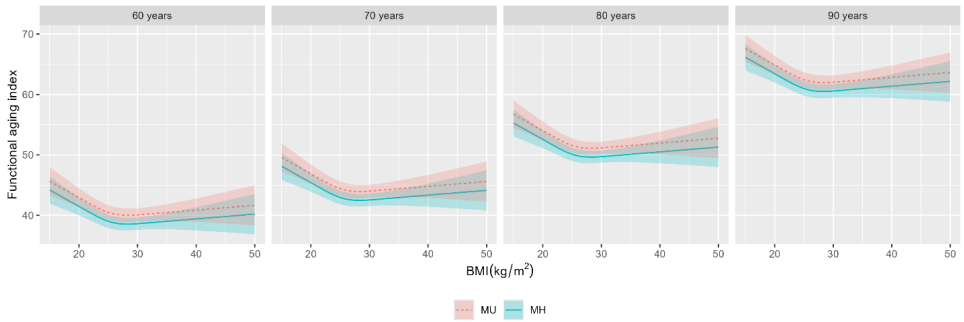

Figure 8. Association between BMI and FAI stratified by MHS across age groups.

Predicted FAI values were derived from mixed-effects model with random intercepts at the twin-pair and individual levels, for females, never smokers with more than basic education. Age and BMI were modeled as restricted cubic splines with three knots. Red dashed lines represent the metabolically unhealthy group, while blue solid lines represent the metabolically healthy group. Shaded areas around each line represent the 95% confidence intervals. Each curve shows the BMI-FAI association at four representative ages, from 60 to 90 years, in 10-year intervals. Figure reproduced from Ler et al., Geroscience 2024.[156]

Abbreviations: MU – metabolically unhealthy, MH – metabolically healthy

4.2.2.2 FI models

In contrast, the FI models revealed significant three-way interactions between BMI, metabolic health, and chronological age, highlighting FI's dependency on all three factors: BMI, metabolic health, and chronological age (p-interaction = 0.006).

In the ages under 65, there was no statistically significant interaction between BMI and MHS (all p-interactions > 0.33). BMI was significantly (Wald test p-value = 2.5E-3) and non-linearly (Wald test p-value = 1.3E-3) associated with FI, with the lowest estimated FI (nadir) at BMI 26.3 kg/m², and FI higher at both lower and higher BMI values (see Figure 9A). The difference in the estimated FI between individuals who were metabolically healthy and unhealthy was slight and not statistically significant (β = -0.24, 95% CI = -1.12 to 0.64, p = 0.59).

In the 65 to 85 age group, a statistically significant interaction existed between BMI and MHS (p-interaction = 0.02). Like the <65 age group, the association between BMI and FI remained statistically significant (Wald test p = 3.0E-05) and non-linear (Wald test p = 2.2E-03), with both low and high BMI associated with higher FI. However, the nadir for the metabolically unhealthy group was higher (28.1 kg/m²) than the metabolically healthy group (26.0 kg/m²). Up to a BMI of 28 kg/m², the expected FI was similar in both groups. Beyond this point, the FI increased more steeply in individuals who were metabolically unhealthy (see Figure 9B).

In the group aged 85 and above, a significant interaction between age (as a linear term) and MHS was found (p-interaction = 0.01). In this group, the expected FI increased by an extra 0.52% (95% CI = 0.11 to 0.93) with each year of age for individuals who were metabolically unhealthy compared to those who were metabolically healthy. A non-significant inverse linear association between BMI and FI was observed (β = -0.15, 95% CI = -0.33 to 0.03, p = 0.09; see Figure 9C). Compared to younger age groups, FI changes in this stratum were modest for the metabolically healthy group but substantial for the metabolically unhealthy especially at higher ages due to the interaction effect (see Figure 9C).

4.2.2.3 Sensitivity Analysis

Sensitivity analyses confirmed the robustness of the findings. Adjustments for differences in the data source (GENDER, OCTO-Twin, and GENDER), the measurement period, and including WHR in the metabolic health definition did not alter the main conclusions.

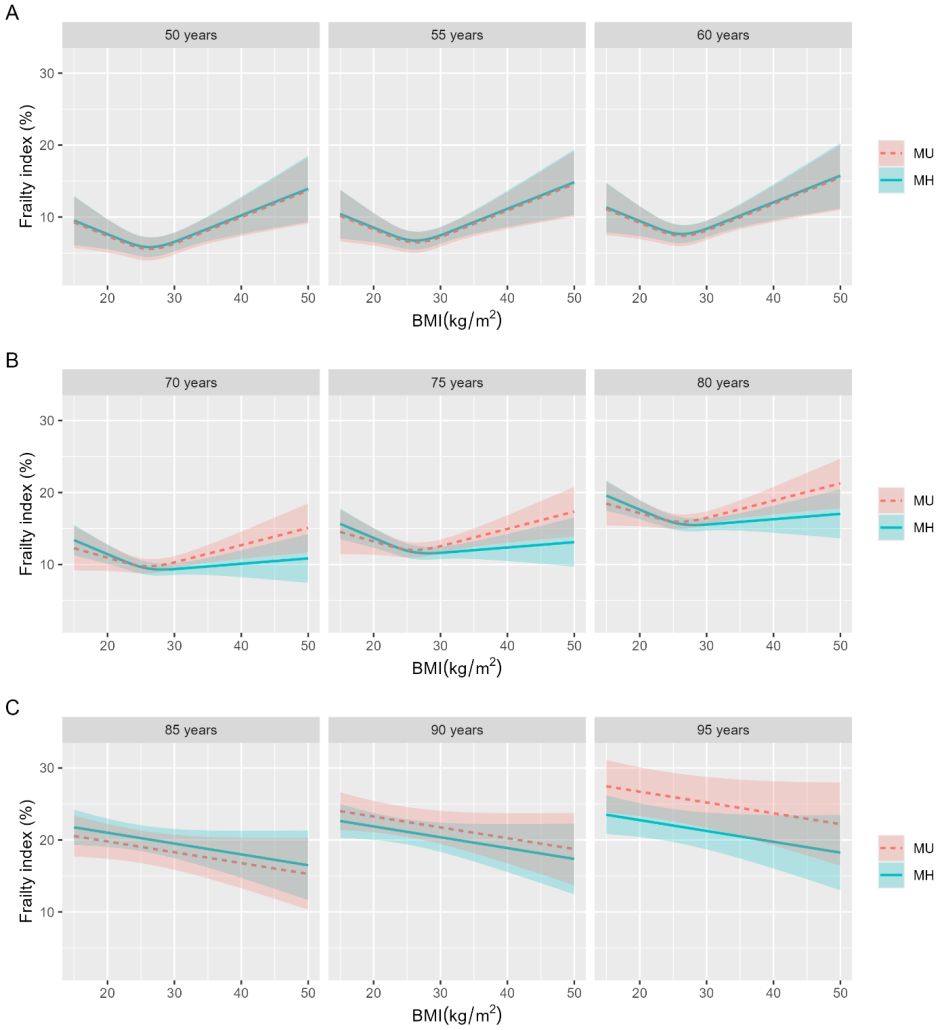

Figure 9. Association between BMI and biological age, measured by FI, stratified by MHS in each age stratum

Predicted FI derived from mixed-effects model with random intercepts at the twin-pair and individual levels for females, never smokers with more than basic education. Age was a linear term except at ages 65 – 85 and modelled as a restricted cubic spline (RCS) with 3 knots. BMI was modeled as RCS with 3 knots, except in ages 85 and above, where it was modelled as a linear term. Red dashed lines represent the metabolically unhealthy group, while blue solid lines represent the metabolically healthy group. Shaded areas around each line represent the 95% confidence intervals. Figure reproduced from Ler et al., Geroscience 2024.[156]

Abbreviations: MU – metabolically unhealthy, MH – metabolically healthy

Findings from Sensitivity Analysis of Individual Metabolic Components Jointly with BMI

Figure 10 presents the models analyzing individual metabolic health components. The nonlinear relationship between BMI and FAI was consistent across all age groups. For FI, this association persisted only in those under 85. For participants 85 and older, the BMI-FI associations were not statistically significant.

With FAI as the outcome, hyperglycemia (β = 3.27, 95% CI = 2.09 to 4.45, p = 7.1E-7), hypertriglyceridemia (β = 1.21, 95% CI = 0.63 to 1.79, p = 4.3E-5), and low HDL-C (β = 1.17, 95% CI = 0.56 to 1.78, p = 2.0E-4) were significantly associated with higher FAI. Hypertension was associated with lower FAI (β = -1.03, 95% CI = -1.71 to -0.34, p = 0.003). Figure 10A — D illustrates the FAI-BMI relationships stratified by each metabolic component.

In the FI models for individuals under 65, hypertension was associated with lower FI (β = -0.89, 95% CI = -1.77 to -0.02, p = 0.05). Other metabolic components showed no significant association in this age group (see Figure 10E — H).

In the 65 — 85 age group, hyperglycemia showed the strongest association with FI, with significant interaction with BMI (p-interaction = 0.003). Individuals with hyperglycemia had a more pronounced increase in FI at low and high BMI than those with normoglycemia (Figure 10J). Hypertriglyceridemia was associated with higher FI (β = 1.09, 95% CI = 0.24 to 1.94, p = 0.01), while hypertension and low HDL were not significantly associated with FI. Interactions between BMI and other metabolic components were not statistically significant (Figures 10I, K, and L).

For participants aged 85 and older, significant interactions were found between chronological age and hyperglycemia (p = 0.001), hypertriglyceridemia (p = 0.01), and low HDL-C (p = 0.003), but not hypertension. In the presence of hyperglycemia, hypertriglyceridemia, and low HDL-C, FI increased by an extra 0.83% (95% CI = 0.32 to 1.34), 0.87% (95% CI = 0.20 to 1.53), and 0.73% (95% CI = 0.25 to 1.21) per year of age increment, respectively (see Figure 10M — P).

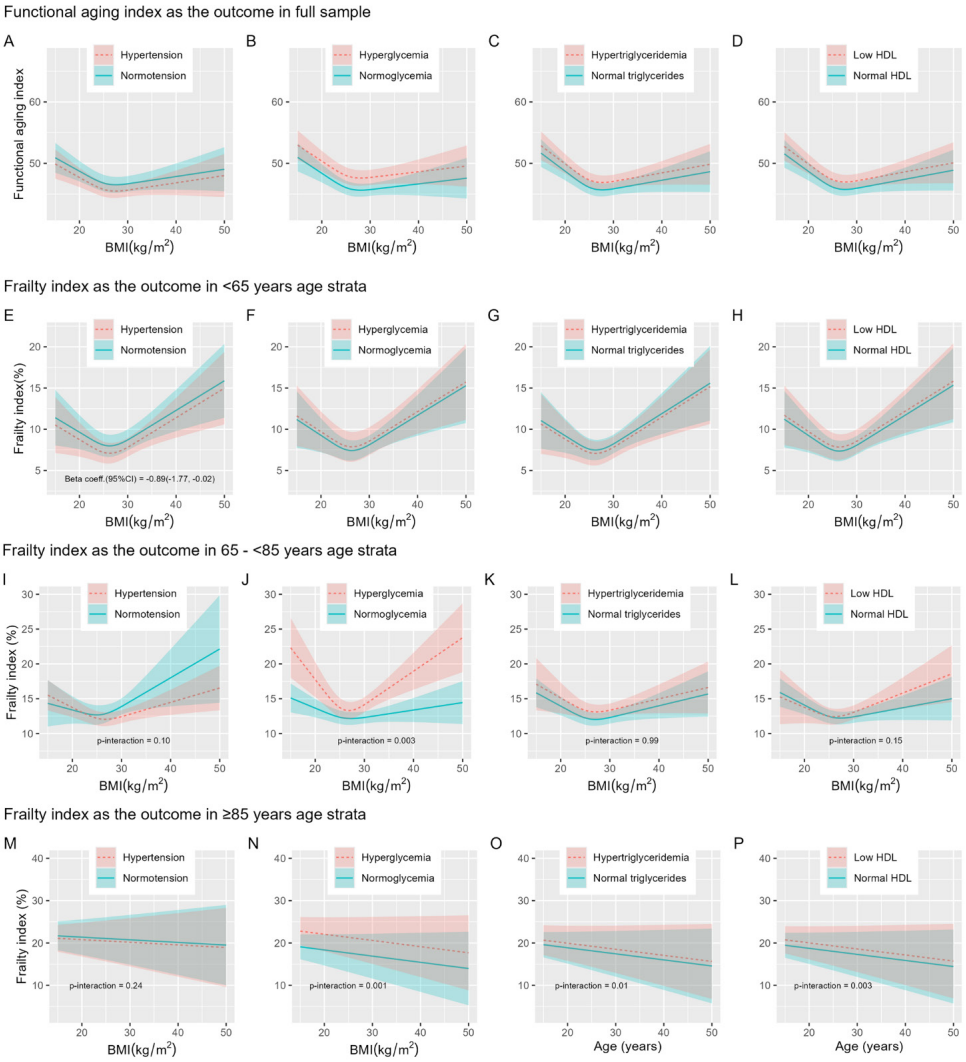

Figure 10. Association between BMI and biological age, measured by FI, stratified by status of individual metabolic component

Predicted FI derived from mixed-effects model with random intercepts at the twin-pair and individual levels and fixed effects for sex, education, and smoking history. Age was a linear term except at ages 65 – 85 and modelled as a restricted cubic spline (RCS) with 3 knots. BMI was modeled as RCS with 3 knots, except for ages 85 and above, where it was modeled as a linear term. Red dashed lines represent the presence of individual metabolic components, while blue solid lines represent the absence of individual metabolic components. Shaded areas around each line represent the 95% confidence intervals. Figure reproduced from Ler et al., Geroscience 2024.[156]

4.2.3 How We Tackled Challenges

Given the significant three-way interaction observed in the FI model, we stratified the analysis to explore further how BMI and MHS jointly influence FI. This stratification resulted in three groups based on changes in predicted FI with age. However, it also reduced the number of observations within each stratum, a common challenge in stratified analyses. Sparse data in specific subgroups, particularly at lower and higher ages, can limit statistical power and increase estimate variability. Although not stratifying by age could have avoided the smaller sample sizes in the youngest and oldest groups, retaining the three-way interaction would have compromised the interpretability of the results. Since the number of observations within each stratum was not too small, we proceeded with stratified analyses.

The data was combined from multiple studies, which enhanced statistical power and allowed us to stratify. However, this approach introduced challenges related to differences in baseline characteristics and data collection periods across the studies. To account for variations across the studies pooled together, we adjusted for study differences by including them as a categorical covariate in sensitivity analyses. Additionally, we included the year of data collection in 10-year intervals in the model as a sensitivity analysis — to account for temporal trends in the prevalence of overweight, obesity, and metabolically unhealthy states.

It is essential to acknowledge the cross-sectional nature of the analyses. Cross-sectional studies provide a snapshot of how these factors interact at a given time, offering a clear overview of their associations across different ages. Given that BMI, metabolic health, and aging are closely intertwined, this cross-sectional perspective is beneficial for identifying patterns and immediate relationships within the population. It can serve as a starting point to generate hypotheses.

However, the cross-sectional design also has limitations. It cannot establish causal links or temporal relations or determine how these relationships evolve, leaving the possibility of reverse causality — whereby aging or deteriorating health may influence BMI and metabolic health rather than vice versa. This limitation indicates the need for further studies examining the underlying mechanisms through longitudinal studies.

4.3 Study III — The Mediating Role of Epigenetic Aging in the Nonlinear Association Between BMI and Mortality

While **Study I** revealed the joint associations of BMI and metabolic health with mortality risk, **Study II** provided a broad snapshot of how low BMI, high BMI, and metabolic health were linked to higher functional and physiological aging and highlighted the complex interaction between BMI, metabolic health, and chronological age. In **Study III**, we shifted the focus from functional and physiological aging to the cellular level, exploring epigenetic aging as a potential mediator in the relationship between BMI and mortality.

4.3.1 How We Got Here: Methods Overview

For this study, we used data from the Health and Retirement Study (HRS), which provided 2016 data on self-reported BMI and EAA measurements from blood samples and mortality follow-up data through December 2020. Figure 11 presents a direct acyclic graph that guided our statistical approach.

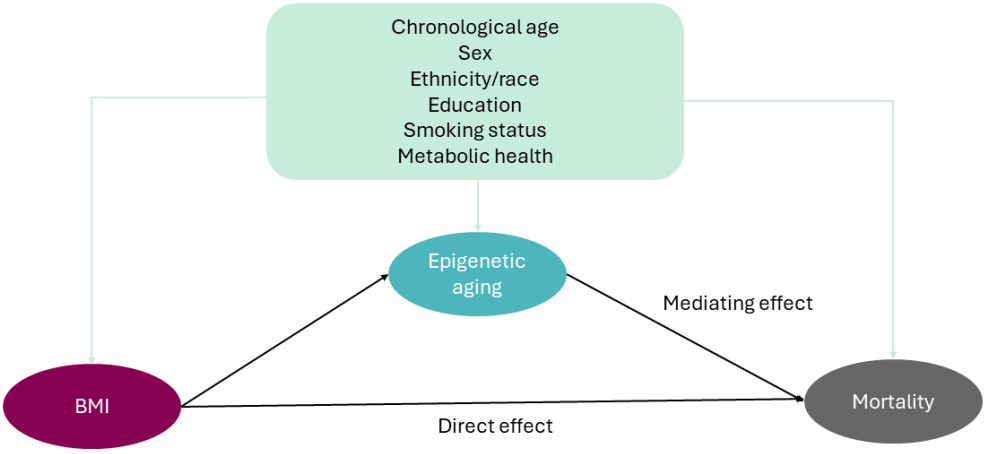

Figure 11: Direct acyclic graph guiding the statistical approach

We performed mediation analysis using the potential outcomes framework (see Chapter 3, section 3.4.5 for details). This allowed us to estimate for the ADE and ACME.

Separate analyses were performed for each EAA measure — HannumAgeAcc, PhenoAgeAcc, GrimAgeAcc, and DunedinPACE — which served as mediators in the relationship between BMI (low and high) and all-cause mortality.

To recap:

- ADE reflects the average difference in outcomes when BMI changes between control (reference) and treated (exposed) levels while EAA is kept constant.
- ACME represents the average difference in mortality outcomes when the mediator (EAA) varies between treatment or exposed (BMI) conditions while keeping the exposure level constant.

The mediation analysis involved specifying two models:

1. Mediator model**:** Linear regression to assess the cross-sectional relationship between BMI and each EAA measure
2. Outcomes model**:** Gompertz proportional hazards model with chronological age as the underlying timescale to evaluate the associations between BMI, EAA, and mortality. The Gompertz distribution was chosen based on the lowest AIC value compared to other parametric survival models.

The estimates from the mediation analysis — ACME and ADE — represent the difference in survival time (ΔST) between the treated (exposed) and control (reference) BMI levels, expressed in years of life gained or lost. In this study, we set the control (reference) level at BMI 27 kg/m², associated with the longest life expectancy. The treatment levels for BMI were 19 kg/m² for low BMI and 35 kg/m² for high BMI.

We adjusted both models for chronological age, sex, race/ethnicity, education attainment, smoking status, and metabolic health. Where appropriate, RCS was applied to continuous variables to account for nonlinear relationships, selected based on AIC

and LRT. We performed 3,000 bootstrap resamples to estimate the 95% confidence intervals for the estimands in mediation analyses.

Study III defined metabolically healthy status as the absence of metabolic abnormalities. As part of the sensitivity analysis, we used a more lenient definition of metabolic health in sensitivity analyses, categorizing participants as metabolically healthy if they had no more than one metabolic abnormality. We also repeated the mediation analysis after excluding follow-ups that were 12 months or less.

4.3.2 What We Found

The study sample consisted of 3,840 individuals aged 51 to 100 years in 2016, with a mean age of 70 (SD = 9.7). Of the participants, 58% were female, 75% identified as White/Caucasian, 17% as Black/African American, and 8% as other ethnicities/races. By the end of 2020, 423 participants (11.0%) were deceased.

4.3.2.1 Mediator models

Our analysis showed linear relationships (see Figure 12A), between BMI and HorvathAgeAcc-I (β = 0.03, 95% CI = -0.003 – 0.07), although this was not statistically significant; and between BMI and HorvathAgeAcc-II (β = 0.04, 95% CI = 0.01 – 0.06).

In contrast, nonlinear relationships were found between BMI and the other EAA measures. Both low and high BMI levels were associated with increased biological aging (see Figure 12C–F). The BMI levels corresponding to the lowest EAA (nadir) were 25.4 kg/m² for HannumAgeAcc, 23.5 kg/m² for PhenoAgeAcc, 25.8 kg/m² for GrimAgeAcc, and 24.7 kg/m² for DunedinPACE.

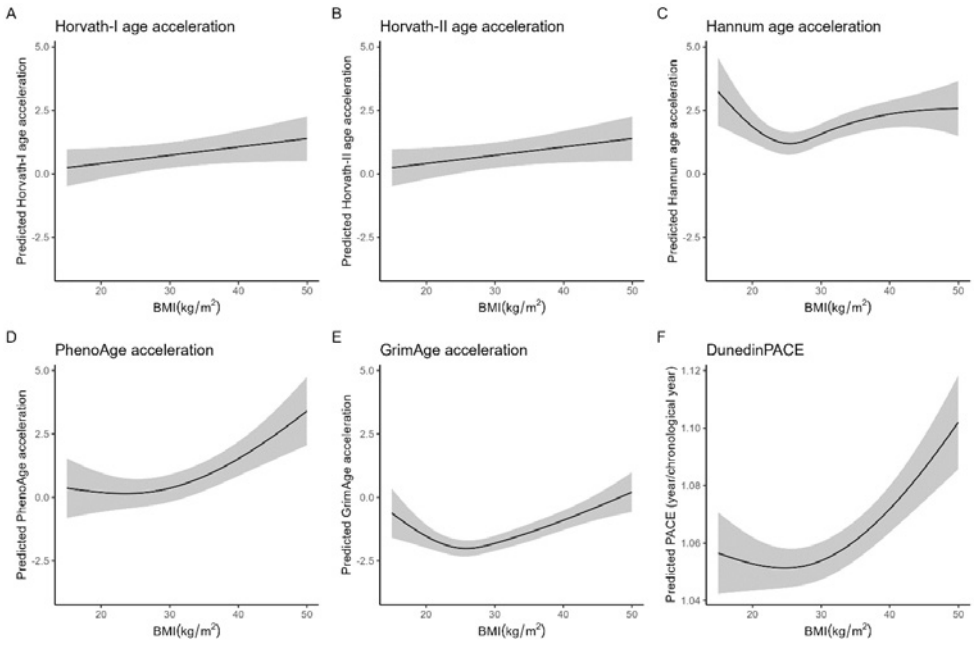

Figure 12. Association Between BMI and Epigenetic Age Acceleration

Predicted estimates were computed from linear models and represent white males aged 70 years who were never smokers, from the metabolically unhealthy group, and had a high school education or lower.

4.3.2.2 Outcome models

BMI displayed a nonlinear relationship with predicted life expectancy, with the shortest life expectancies observed at both extremes of BMI. The BMI associated with the longest life expectancy was approximately 27 kg/m² (see Figure 13A).

Figures 13B–G depict predicted mean life expectancy as a function of EAA, adjusted for white males aged 70, who were metabolically unhealthy, never smoked, had a high school education or lower, and had a 25 kg/m² BMI. Contrary to expectations, HorvathAgeAcc-I and HorvathAgeAcc-II showed concave associations with survival, where lower EAA was linked to reduced life expectancy (Figures 13B & C).

HannumAgeAcc followed a similar pattern, though lower EAA values were generally associated with higher life expectancy (Figure 13D).

In contrast, PhenoAgeAcc, GrimAgeAcc, and DunedinPACE demonstrated a consistent decline in predicted life expectancy as EAA increased (Figures 13E–G). - A one standard deviation increase in PhenoAgeAcc, GrimAgeAcc, and DunedinPACE was associated with a 32% (HR = 1.32, 95% CI = 1.23 – 1.51), 72% (HR = 1.72, 95% CI = 1.50 – 1.88), and 40% (HR = 1.40, 95% CI = 1.25 – 1.56) higher risk of mortality, respectively.

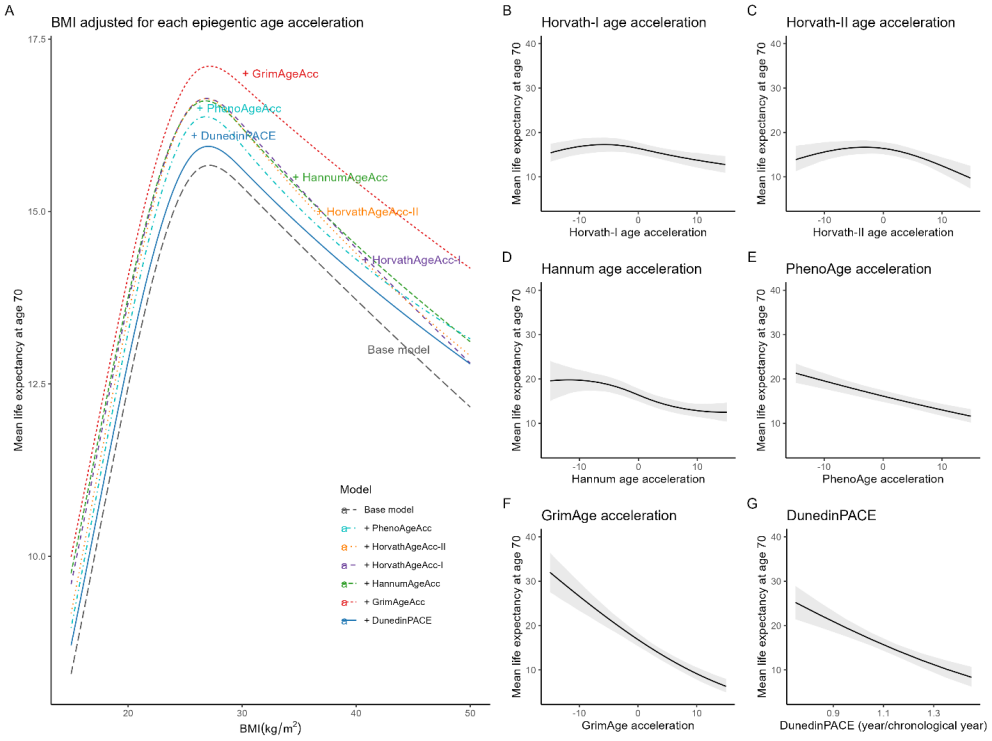

Figure 13: Association Between Body Mass Index and Epigenetic Age Acceleration Measures with Mean Life Expectancy

Mean life expectancies were predicted from the Gompertz proportional hazards model, using chronological age as the underlying timescale, and represent white males aged 70, with average levels of either BMI (panel A) or EAA (panel B – G), who were never smokers, had a high school education or less, and in the metabolically unhealthy group.

4.3.2.3 Epigenetic Age Acceleration as a Mediator

The mediation analysis revealed that EAA partially mediated the relationship between BMI and mortality (see Figure 14). For individuals with higher BMI, EAA accounted for a significant portion of the association with reduced survival time. In this group, the ADE on survival, independent of EAA, showed that BMI at 35 kg/m^2 was associated with 1.22–1.58 years shorter survival than BMI at 27 kg/m^2. Notably, ADEs of high BMI were not statistically significant. The ACME, representing the indirect or mediating effect through EAA, was smaller but statistically significant, contributing to approximately 0.28 – 0.72 year reduction in survival time. This indicates that EAA mediates 15 – 37% of the total association between high BMI and survival.

Conversely, the direct effects on mortality were more pronounced and statistically significant for lower BMI. A BMI of 19 kg/m^2 was associated with a 5.60 – 6.38 years shorter survival time than a BMI of 27 kg/m^2. The mediated effect through EAA was relatively modest in comparison, accounting for only 7 – 11% of the total association between lower BMI and survival time, with the ACME statistically significant when the EAA was either HannumAgeAcc [predicted difference in survival time (ΔST) = 0.44, 95% CI=-0.87 – -0.10] or GrimAgeAcc (ΔST = 0.74, 95% CI=-1.44 – -0.16).

Sensitivity analyses adjusting the models using a less strict definition of metabolic health and limiting the analytical sample to those who survived more than 12 months had little impact on the results (see Figure 15).

A note on Metabolic Health

Although metabolic health was not a main exposure of interest specifically in this study, the presence of any metabolic abnormality was associated with higher EAA measured as HorvathAgeAcc-II (β=0.46, CI=0.08 - 0.85), HannumAgeAcc (β= 0.68, CI=0.22 – 1.13), PhenoAgeAcc (β=1.30, CI=0.70 – 1.90), GrimAgeAcc (β=1.29, CI=0.96 – 1.62), and DunedinPACE (β=0.02, CI=0.01 – 0.02). Metabolic health was not significantly associated with survival outcomes.

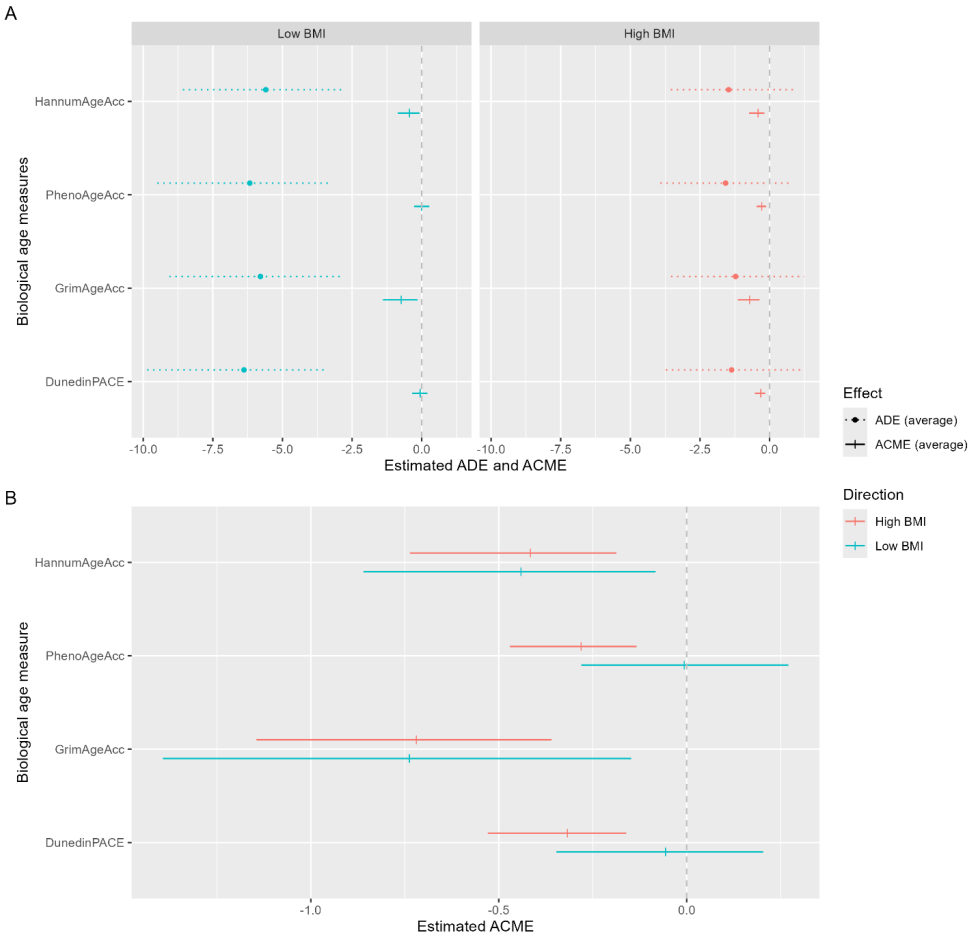

Figure 14: Estimated ADE and ACME by Epigenetic Age Acceleration in the BMI-Mortality Association

This figure shows the mediation analyses estimating the average direct effects (ADE) and average causal mediation effects (ACME) for the association between BMI and survival, with epigenetic age acceleration as the mediator. Linear models assessed the BMI-mediator relationship, and Gompertz proportional hazards models were used for survival outcomes. Adjustments were made for sex, race/ethnicity, smoking, education, and metabolic health. The x-axis shows the mean difference in survival time.

- Panel A: The left panel shows ADE (dotted lines) and ACME (solid lines) for low BMI (19 kg/m² vs. 27 kg/m²), and the right panel for high BMI (35 kg/m² vs. 27 kg/m²).
- Panel B: Shows ACME for high BMI (blue) and low BMI (red).

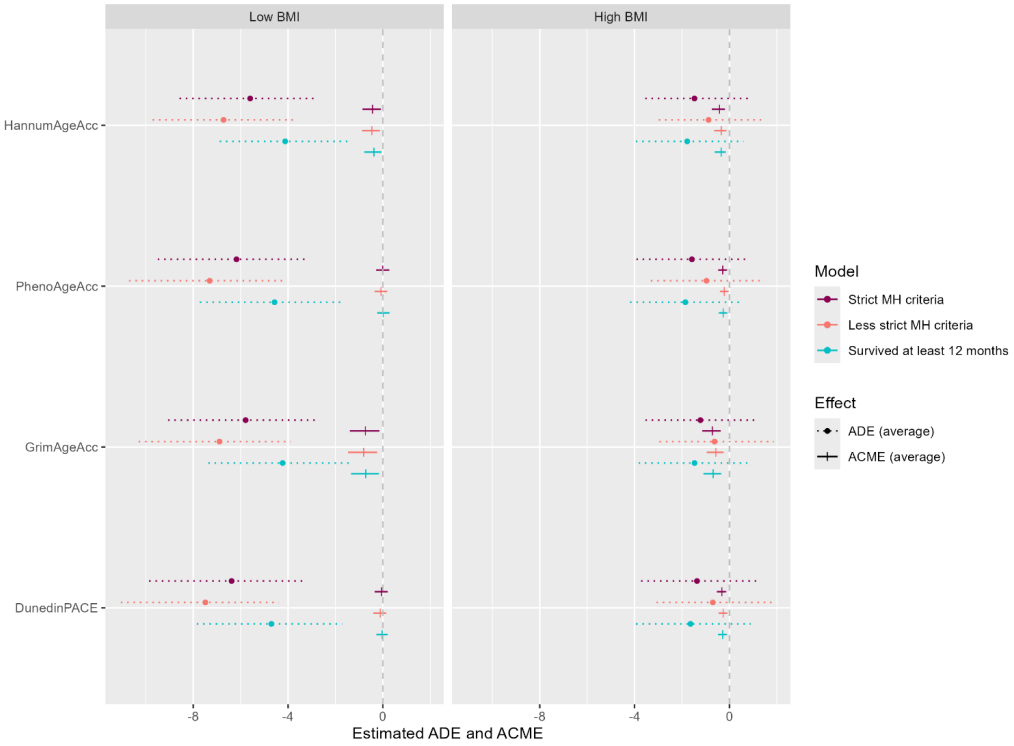

Figure 15: Estimated ADE and ACME by Epigenetic Age Acceleration in the BMI-Mortality Association from Sensitivity Analysis

This figure shows ADE and ACME estimates from mediation analyses of BMI and survival, with epigenetic age acceleration as the mediator adjusted for metabolic health defined with a strict criteria (no hypertension, hyperglycemia, hypertriglyceridemia, or low HDL) used in the main analysis, a less strict criteria (no more than one condition), and mediation analysis on a sample including only those who survived at least 12 months. The x-axis represents the mean difference in survival time.

- The left panel shows ADE (circles) and ACME (cross) for low BMI (19 kg/m² vs. 27 kg/m², in blue), using the strict criteria (solid lines), less strict criteria (dotted lines) and sample excluding deaths in the first 12 months (dashed lines)
- The right panel shows ADE (circles) and ACME (cross) for high BMI (35 kg/m² vs. 27 kg/m², in red), using the strict criteria (solid lines), less strict criteria (dotted lines) and sample excluding deaths in the first 12 months (dashed lines)

4.3.3 How We Tackled Challenges

By treating BMI as a continuous variable, we reduced the loss of information from categorization or stratification, allowing for evaluating both low and high BMI as contributors to mortality. Additionally, we accounted for nonlinearity in the associations by applying restricted cubic splines, enabling us to explore more complex relationships.

Despite these strengths, there were limitations. BMI was calculated using self-reported height and weight, which may introduce measurement errors. While self-reported BMI has been shown to closely approximate measured BMI,[157, 158] we should consider potential biases such as under-reporting in individuals with higher BMI and over-reporting among those with lower BMI. These discrepancies could have influenced the accuracy of our findings.

The relatively short follow-up period (2016–2020) restricted our capacity to observe long-term mortality outcomes, which may limit the robustness of the associations observed. Extending the follow-up period would have provided a more accurate assessment of the long-term effects of BMI and EAA on survival.

The study employed a prospective design by considering 4-year mortality as the outcome, but BMI and EAA were measured cross-sectionally in 2016. This limited our ability to draw causal inferences between BMI, biological aging, and survival outcomes. Notably, the possibility of reverse causality — where accelerated biological aging could affect BMI, especially in older individuals — cannot be ruled out. Data on BMI and EAA at multiple time points could help clarify the temporal relationships between these factors.

4.4 Study IV — ΔBMI and ΔBiological Age: Which came first?

Study IV aimed to disentangle the temporal dynamics of how BMI and biological aging influence each other. The goal was to assess whether a change in BMI over time predicts changes in biological aging, as measured by functional and physiological aging markers, and conversely, whether changes in biological aging predict a change in BMI.

4.4.1 How We Got Here: Methods Overview

We used longitudinal data on BMI and biological aging measures (FAI and FI) from GENDER, OCTO-Twin, and SATSA. DCSM (see Chapter 3, section 3.4.7 for details) was applied following the workflow illustrated in Figure 16. We began by dividing the data into two-year age bins using chronological age as the underlying timescale. Age bins consisting of less than 100 assessments of BMI and biological aging measures were excluded from the analysis, resulting in age bins covering ages 60.0 to 91.9 years. Then, the BMI, FAI, and FI age trajectories were estimated through univariate DCSM. Subsequently, bivariate DCSM models were applied to examine the longitudinal association between BMI and BA (FAI and FI), allowing us to estimate if the association between change in BMI and biological aging is bidirectional or unidirectional.

We adjusted all models for sex, smoking status, study, and twin-relatedness.

4.4.2 What We Found

In the FAI and FI analyses, 1,902 participants had at least one BMI or FAI measurement, and 1,976 participants had at least one BMI or FI measurement. Of these, 1,207 had at least three BMI or FAI measurements, and 1,291 for BMI or FI measurements.

The mean age for both the FAI and FI analytical samples was similar, approximately 74 (SD= 8), and the mean BMI was 25.6 kg/m^2 (SD=4.0). The mean FAI was 48.0 (SD=11.7), and the mean FI was 15.7% (SD = 10.3). In both samples, about 59% were females, and 52% were never smokers. The proportion of the analytical sample from GENDER, OCTO-Twin, and SATSA was slightly different in the FAI (26%, 31%, and 43%, respectively) and FI samples (25%, 33%, and 41%, respectively).

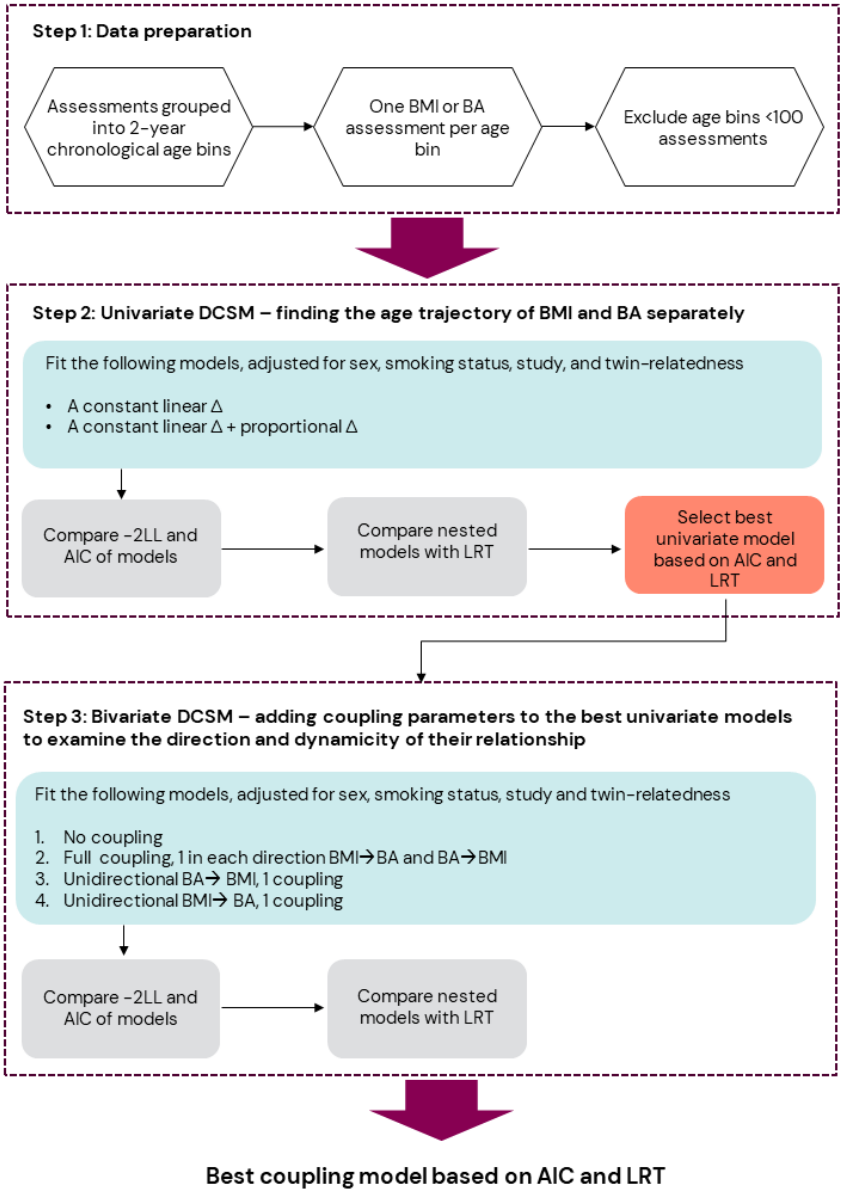

Figure 16: Statistical analytical workflow for **Study IV**

Abbreviations: BA – biological aging measure, BMI – body mass index

4.4.2.1 BMI, FAI, and FI age trajectories

In the univariate models of changes in BMI, FAI, and FI, the model with a proportional change parameter offered a better fit than one with only the constant change parameter. BMI had a negative linear slope offset by a positive proportional effect, resulting in a decline from ages 60 to 91.9. For FAI and FI, changes were similarly driven by a negative linear slope and a positive proportional change, leading to an exponential increase in FI and FAI as the participants aged.

4.4.2.2 Bivariate DCSM

For BMI and FAI, the best-fitting model was unidirectional, with FAI changes preceding changes in BMI. Including coupling from FAI reduced both the linear slope and proportional change in BMI but with a negative coupling parameter, indicating that higher FAI was associated with a greater decline in BMI (Table 8). As FAI levels were lower in younger ages and increased exponentially with age, the coupling effect led to a slight increase in BMI at younger ages, followed by a leveling off and then a steeper decline in BMI at older ages (see Figure 17).

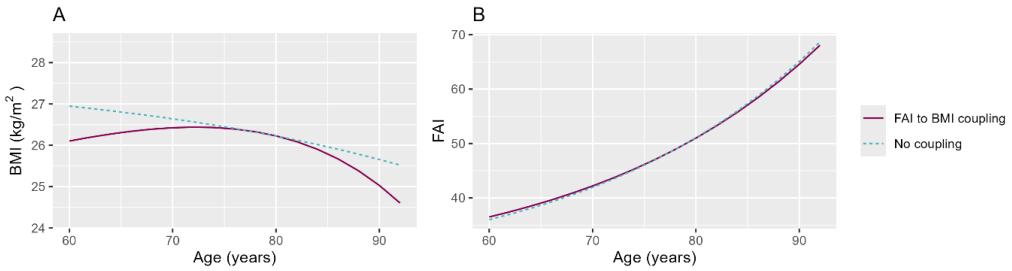

Figure 17: BMI and FAI trajectories (A & B) from bivariate dual change score models with and without coupling effects

Blue dotted lines represent trajectories without coupling, while red lines show those with coupling effects. Trajectories were generated from ages 60 to 91.9 using bivariate dual change score models, adjusted for age, sex, smoking, study, and twin-relatedness. The model was unidirectional, with one coupling parameter from FAI to BMI.

Abbreviations: BMI – body mass index, FAI – functional aging index

Table 8: Longitudinal association between BMI and FAI from Dual Change Score Models

	No coupling		FAI to BMI coupling	
	Estimate	**95% CI**	**Estimate**	**95% CI**
Mean intercept BMI (μBMI_0)	26.95	26.37,27.52	26.10	25.47,26.74
Mean slope BMI (μBMI_{slope})	-1.77	-2.67,-0.87	-0.69	-1.57,0.19
Mean intercept FAI (μFAI_0)	35.99	33.85,38.13	36.49	34.54,38.43
Mean slope FAI (μFAI_{slope})	-2.23	-3.47,-0.99	-2.35	-3.53,-1.17
Proportional change parameters (β)				
β_{BMI}	0.06	0.03,0.10	0.05	0.02,0.08
β_{FAI}	0.09	0.07,0.11	0.09	0.07,0.11
Coupling parameters (γ)				
$\gamma_{FAI \rightarrow \Delta BMI}$			-0.02	-0.02,-0.01

Estimates and 95% confidence intervals were derived from dual change score models with and without coupling parameters. μBMI_0 and μBMI_{slope} denote mean BMI intercept and slope; μFAI_0 and μFAI_{slope} denote mean FAI intercept and slope; β_{BMI} and β_{FAI} denote proportional change of BMI and FAI; $\gamma_{FAI \rightarrow \Delta BMI}$ denotes coupling parameters from FAI to BMI change.

Abbreviations: BMI – body mass index, FAI – functional aging index

For BMI and FI, the best-fitting model revealed a bidirectional association. Like FAI, when coupling from FI was added, both the linear slope and proportional change in BMI decreased compared to the no-coupling model (see Figure 18). A negative coupling parameter indicated that higher FI was associated with a greater decline in BMI (see Table 9). Since FI levels were lower in younger individuals and rose exponentially with age, the coupling effect led to a slight increase in BMI at younger ages, stabilization around ages 70 to 75, and a more pronounced decline at older ages.

For FI, incorporating coupling from BMI reduced the initial intercept, strengthened the negative linear slope, and weakened the proportional change while introducing a positive coupling effect from BMI (Table 9). As BMI decreased with age, this contributed to a lower initial FI level but led to a sharper rise in FI up until approximately age 75, followed by a less steep increase after age 85, compared to the no-coupling model (Figure 18B).

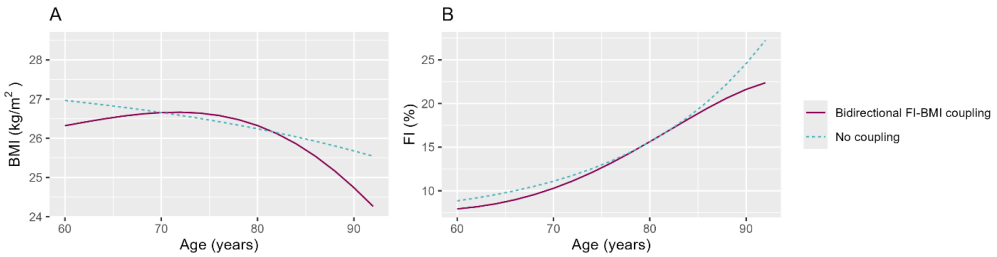

Figure 18: BMI and FI trajectories (A & B) from bivariate dual change score models with and without coupling effects

Blue dotted lines represent trajectories without coupling, while red lines show those with coupling effects. Trajectories were generated from ages 60 to 91.9 using bivariate dual change score models, adjusted for sex, smoking, study, and twin-relatedness. Model with coupling was bidirectional, with one coupling parameter from FI to BMI, and one from BMI to FI.

Abbreviations: BMI – body mass index, FI – frailty index

Table 9: Longitudinal association between BMI and FI from Dual Change Score Models

	No coupling		Bidirectional coupling	
	Estimate	**95% CI**	**Estimate**	**95% CI**
Mean intercept BMI (μBMI_0)	26.97	26.4,27.53	26.32	25.76,26.88
Mean slope BMI (μBMI_{slope})	-1.75	-2.58,-0.92	-0.36	-1.37,0.65
Mean FI intercept (μFI_0)	8.83	7.37,10.29	7.91	6.41,9.42
Mean FI slope (μFI_{slope})	-0.97	-1.47,-0.47	-23.44	-28.89,-17.99
Proportional change parameters (β)				
β_{BMI}	0.06	0.03,0.09	0.03	-0.01,0.07
β_{FI}	0.15	0.12,0.18	0.14	0.11,0.17
Coupling parameters (γ)				
$\gamma_{BMI \text{ -> } \Delta FI}$			0.86	0.65,1.06
$\gamma_{FI \text{ -> } \Delta BMI}$			-0.04	-0.05,-0.03

Estimates and 95% confidence intervals were derived from dual change score models with and without coupling parameters. μBMI_0 and μBMI_{slope} denote mean BMI intercept and slope, μFI_0 and μFI_{slope} denote mean FI intercept and slope; β_{BMI} and β_{FAI} denote proportional change of BMI and FI; $\gamma_{FI \rightarrow \Delta BMI}$ denotes coupling parameters from FI to BMI change, $\gamma_{BMI \rightarrow \Delta FI}$ denotes coupling parameter from BMI to FI

Abbreviations: BMI – body mass index, FI – frailty index

4.4.3 How We Tackled Challenges

The limitation of pooling data from three distinct longitudinal cohorts, which may introduce heterogeneity, is also present in this study, like in **Study I** and **II**. Participants in the GENDER and OCTO-Twin studies were older at baseline than those in the SATSA study. Additionally, each cohort entered the analysis at different starting ages: SATSA at 60, GENDER at 70, and OCTO-Twin at 80. This could lead to survival bias, as the twins in the GENDER and OCTO-Twin studies were likely healthier to have been included at

older ages. Models were adjusted for cohort membership to account for this, though survival bias could not be fully eliminated.

Although DCSMs can be adjusted for confounders such as sex and smoking, the trajectories for males and females, smokers and non-smokers, may differ. Consequently, these sub-groups could exhibit varying BMI-biological age relationships. A limitation of this study is the inability to stratify the DCSMs by these factors, as the small sample size made such stratifications infeasible.

In our study, we assumed no variation in proportional change across different ages. Although some models incorporate breakpoints to allow proportional change and coupling effects to vary by age, attempts to include breakpoints in this study led to model instability. Therefore, we did not include breakpoints, which limited the ability to capture turning points in trajectories and age differences in the associations. Combining the estimates for a constant linear change component and a proportional change component leads to variations in intercepts, slope coefficients, and a proportional change parameter, typically resulting in an exponential trajectory with either an increasing or decreasing trend.[147]

While it is unlikely that trajectories in biological aging measured at three-year intervals will have turning points, BMI has been shown to exhibit fluctuations, such as an increase in midlife (ages 50 to 65), stabilization, and a decline around age 80.[49] It is possible that models without breaks in proportional change inadequately capture such variations. Extensive testing was performed by including a wider age range and breakpoints in the proportional change and coupling effects, but these tests are not reported here due to model instability. Despite this, the overall direction of the association between BMI and FAI or FI remained consistent. Future research should investigate the causes of instability when including breakpoints to proportional change within DCSM.

5 Weighing the Evidence

5.1 Summary of Results

In this thesis, I attempted to unravel the complex relationships between BMI, metabolic health, biological aging, and all-cause mortality. Through four studies, we explored how BMI and metabolic health, independently, jointly, and in interaction, associate with biological aging and survival, each offering unique insights into the interplay between BMI, metabolic health, aging, with health outcomes. Below is a synopsis of the findings:

Study I: Metabolically unhealthy status in midlife and late life was consistently associated with increased mortality risk, even after adjusting for BMI. Metabolically unhealthy obesity in midlife, as well as being metabolically unhealthy across all BMI categories in late life, were associated with increased mortality risk. In late life, metabolically healthy and overweight had a lower risk of mortality, though this inverse association was not observed in midlife.

Study II: Both low and high BMI and metabolically unhealthy status were associated with higher functional and physiological aging. The effect modification of BMI by metabolic health was significant only when biological aging was measured by FI, particularly in individuals aged 65 to 85. In addition, metabolic health modified the association between chronological age and FI, with the metabolically unhealthy group associated with a greater increase in FI with each advancing year in ages 85 and above.

Study III: Both low and high BMI levels were associated with greater epigenetic age acceleration and shorter life expectancy. Our findings suggest that epigenetic aging partially mediated the relationship between high BMI and survival, contributing to shorter survival times. In contrast, the evidence of epigenetic aging as a mediator in the low BMI-survival relationship was weaker, with low BMI showing stronger direct effects on shorter survival time.

Study IV: The relationship between BMI and FAI was unidirectional, with functional aging driving changes in BMI. However, the relationship between BMI and FI was bidirectional, with BMI influencing physiological aging and vice versa. These results highlight the dynamic interactions between BMI and aging, depending on the

specific measure of biological aging and how they contribute to health outcomes over time.

These studies offer a more nuanced understanding of how BMI, metabolic health, and biological aging potentially contribute to health and longevity.

5.2 The Weight of the Extremes

Synthesizing the findings from all **Study I** to **III**, a clear nonlinear association between BMI and biological aging or mortality risk emerged. Both low and high BMI were associated with adverse health outcomes, forming a U-shaped — or sometimes as J-shaped — curve. These associations are well documented in previous research, which has consistently shown that extremes in BMI increase the risk of fraillty[96] and mortality in the general population,[e.g.8-10, 32, 34, 37, 38], and among older adults.[159]

While **Study I** was not explicitly designed to explore the non-linearity of BMI, it revealed that individuals with obesity (BMI ≥ 30 kg/m²) in mid and late life had a higher risk of mortality compared to those with normal weight, aligning with previous findings which showed high BMI was associated with increased all-cause mortality.[e.g.8-10, 32, 34, 37, 38] Importantly, a history of overweight or obesity in midlife increased mortality risk in late life regardless of late-life BMI, reinforcing the established risk attributable to high BMI in midlife. Based on a U.S. cohort, **Study III** further confirmed the U-shape association where low and high BMI were associated with mortality risk.

The same nonlinear relationship between BMI and mortality risk was observed in the BMI-biological aging relationship. **Studies II** and **III** demonstrated that higher and lower BMI levels were associated with higher functional, physiological, and epigenetic aging, building on a limited body of research where BMI's nonlinear form was not previously explored, except concerning frailty risk.[96, 99]

5.2.1 The Middle 'Weigh' — An Optimal BMI in Older Adults?

Interestingly, the nadir for the lowest biological aging or highest life expectancy, found in **Study II** and **Study III**, ranged between BMI 25 and 28, and thus within the overweight range rather than the typical normal weight range recommended for the general population (18.5–25.0).[61] This aligns with other cohort studies showing that the optimal BMI for survival in older adults is between 25 and 30.[13, 160] This

raises the question: could being overweight be less harmful than it seems for older individuals?

While weight loss through restrictive diets is a common intervention for obesity, such diets in older adults, particularly those over age 75, can exacerbate malnutrition among older individuals and increase the risk for conditions related to malnutrition.[160] Therefore, current guidelines urge caution when introducing restrictive diets to older adults and recommend prioritizing physical activity over weight reduction for those who are overweight or obese.[161] In light of **Studies I** and **II**'s findings, metabolic health status may provide additional insights for tailoring interventions in older adults with overweight or obesity.

Together, these studies reveal that BMI's impact on aging is not straightforward. Both ends of the BMI spectrum are associated with poorer health outcomes, manifesting as increased and accelerated biological aging or reduced life expectancy. This nonlinear association underscores the need to view low and high BMI as important risk factors in aging research and health interventions.

5.3 Fitting the Metabolic Piece in the BMI and Aging Puzzle

Findings from **Study I** to **Study III** highlight metabolic health's pivotal role in biological aging and survival outcomes. However, many previous studies may not have considered BMI alongside metabolic health in the same model.[64-66, 93, 97, 98, 102, 110, 111, 113]

Metabolic health emerged as potentially having a more substantial influence on mortality risk and biological aging than BMI based on **Studies I** and **II**. Notably, **Study I** showed that metabolically unhealthy phenotypes across all BMI categories in late life were associated with significantly higher mortality risk compared to MHN. Furthermore, late-life MUN had a higher mortality risk than those with a higher BMI but better metabolic health, a finding consistent with other studies.[162] In contrast, late-life MHOw, and midlife or late-life MHO may not have a higher mortality risk than MHN.

Study II provided further evidence that a metabolically unhealthy status is linked to increased functional and physiological aging, consistent with earlier studies showing a positive association between MetS and frailty risk.[97, 163, 164] There were significant modifying effects of metabolic health on physiological aging, demonstrating that metabolically unhealthy status, when combined with higher

BMI, was associated with higher physiological aging compared to their metabolically healthy counterparts, particularly among individuals aged 65 to 85. Metabolic unhealthy status further amplified the increase in physiological aging with advancing chronological age, particularly after 85, suggesting that metabolic health may have an even more substantial impact on health outcomes, especially in late life.

Although metabolic health was not the main focus and was included in the models as a covariate in **Study III**, being metabolically unhealthy was associated with higher epigenetic aging, aligning with previous studies.[93, 102, 110-113]

In short, metabolic health may play a critical role in aging and survival outcomes, potentially exerting a more substantial influence than BMI alone. Therefore, metabolic health and BMI may be crucial targets for intervention in aging research and healthcare strategies.

5.3.1.1 Lack of Standardized Definitions

However, despite the clear importance of metabolic health, the lack of a consistent and universally accepted definition of metabolic health status presents significant challenges.[15, 72] Different studies use varying criteria to define metabolic health, with thresholds for glucose levels, blood pressure, cholesterol, and triglycerides often differing. These inconsistencies complicate comparisons across studies. Although in **Studies I** to **III**, altering the definition of metabolic health during sensitivity analyses affected the magnitude of the effects rather than the direction, standardized criteria still need to be used to examine how metabolic health impacts aging and mortality.

5.3.2 Not all BMIs are Born Equal?

Adding to this complexity is the metabolic heterogeneity within BMI phenotypes. **Study I** revealed that different metabolic health factors were associated with mortality risk in distinct ways. Specifically, mid and late-life hyperglycemia had the strongest association with increased mortality risk, consistent with previous literature,[80, 165] while hypertension was not significantly associated with risk of mortality, highlighting the nuanced roles of various metabolic components.

Similarly, **Study II** showed that BMI and metabolic health interact differently depending on the specific metabolic factor. For example, metabolically unhealthy

status strengthened the positive link between high BMI and frailty index in the 65–85 age group. However, individual factors like hypertension, hypertriglyceridemia, and low HDL did not have similar effects, with hyperglycemia emerging as the main driver. Moreover, in individuals aged 85 and above, although metabolically unhealthy status and age had a synergistic influence on FI, hypertension did not show the same modifying influence when considered individually. These findings underscore the importance of viewing metabolic health as a collection of distinct factors, each potentially influencing aging and mortality through different mechanisms.

Altogether, these results emphasize that metabolic health is not a uniform construct but a complex interplay of factors that may need to be considered individually. The individual metabolic factors appear to have distinct influences, and their combined effect within the construct of metabolic health could lead to a compounded risk that may not be fully explained by simply the sum of its parts. These findings underline the importance of metabolic health as a whole and through its components to capture its actual impact on health.

5.3.3 A Word on the Metabolically Healthy Obesity Conundrum

The hypothesis that MHO may confer health benefits[15, 17, 69] was not substantiated by findings in **Study I** — although the risk of mortality in MHO was slightly raised, it was not significantly different from the risk for MHN. Nevertheless, the estimated mortality risk appeared lower in MHO than in metabolically unhealthy counterparts — MUO and MUOw. This contrasts with meta-analyses, which have shown significant associations between MHO and increased risk for CVD[22] and all-cause mortality compared to MHN.[20]

Although MHO has garnered much attention and debate, the MUN phenotype may be even more critical.[68, 79] MUN may be a 'silent' high-risk phenotype that goes unnoticed without metabolic health assessments. This underscores the importance of evaluating metabolic health across all BMI categories, particularly in older adults.

However, metabolic health-BMI phenotypes are not static. Individuals can transition between different metabolic health-BMI categories over time, and these changes influence health risks.[15, 29, 47, 69, 71, 81] For example, transitioning from a metabolically unhealthy to a healthy state reduces the risk of illnesses.[155] If weight

history was significantly associated with all-cause mortality risk in **Study I**, suggesting that long-term obesity may increase the likelihood of metabolic dysfunction and poorer health outcomes, then perhaps the history of metabolic health-BMI phenotype should also be a key determinant of health risk. A recent analysis of SATSA data conducted alongside this thesis highlighted the fluctuation of BMI-metabolic health phenotypes over 30 years, further underscoring how dynamic these states can be.[154] This transience complicates our understanding of the interaction between BMI and metabolic health throughout life. Nonetheless, it emphasizes the need to account for the trajectories of BMI and metabolic health over time, rather than relying on static measurements, to fully grasp their effects on aging and mortality.

Despite the evidence that MHO carries higher risks of CVD and mortality compared to MHN,[22] and given its transient nature, we are left with the question: Does a true MHO phenotype exist? Genome-wide association studies (GWAS) have already been conducted to identify genetic variants that define subtypes of obesity, including those that increase adiposity with favorable or unfavorable cardiometabolic effects.[166, 167] These insights help disentangle the mechanisms that separate excess adiposity from its usual metabolic complications. A Mendelian randomization study found that while favorable adiposity reduces the risk of metabolic diseases such as T2DM and CVD, it still increases the risk of non-metabolic conditions like osteoarthritis and venous thromboembolism due to mechanical effects.[168] The verdict on MHO, or favorable adiposity, remains uncertain.

5.4 The Obesity Paradox: A Heavier Dilemma?

The obesity paradox, as highlighted in the introduction, refers to the counterintuitive finding that, particularly in older adults, higher BMI levels are sometimes associated with lower mortality risk.[40] None of the studies included in this thesis show results reflecting this paradox.

In **Study I**, the obesity paradox was not directly observed. Instead, individuals with obesity in both midlife and late life generally had an increased risk of mortality. However, the findings raised the question of whether metabolic health might contribute to the paradox. For example, metabolically healthy individuals who were overweight in late life showed a lower mortality risk, while those with metabolically unhealthy normal weight had higher risks. This suggests that the

protective effects sometimes attributed to a high BMI may be linked to metabolic health rather than BMI itself. A study population with a higher BMI but better metabolic health might create the appearance of a paradox, while the opposite holds for populations with poor metabolic health.

Nonetheless, if metabolic health explains the obesity paradox, it would likely require a substantial portion of the study population with obesity to be metabolically healthy.[169] In **Study I**, about 36% of individuals with obesity were metabolically healthy, and just 5% of the total population had MHO. This makes it unlikely that metabolic health alone can account for the obesity paradox seen in other studies.[169]

Among individuals aged 85 and older in **Study II**, higher BMI was associated with lower physiological aging, though not statistically significant, potentially signaling an obesity paradox. Nevertheless, since this was only observed in the oldest stratum of the analysis, the weak association between BMI and physiological aging in late life is likely explained by selective survival.

The findings in **Study IV** suggest that much of the obesity paradox can be explained by reverse causality. Based on **Study IV**, the mean BMI declined from 60 to 91.9, with both functional and physiological aging driving a steeper BMI decline at older ages. These findings support the explanation that the link between low BMI and poorer health outcomes is due to health deterioration leading to unintentional weight loss rather than a protective effect of higher body weight. As individuals age, unintentional weight loss — often due to underlying acute and chronic health conditions — becomes more common.[161, 170] This may produce a positive association between high BMI and better health outcomes, fabricating an appearance of a protective effect for high BMI simply because lower BMI reflects pre-existing health deterioration.[40-42]

Taken together, these findings support the notion that the obesity paradox may not represent a true protective effect of high BMI but instead reflect the complex interactions between aging and unintentional weight loss.[40-42] As age advances, it becomes increasingly crucial to disentangle the role of BMI from the physiological processes that drive health outcomes, particularly unintentional weight loss and metabolic dysfunction, which may mask the actual risks associated with higher BMI in older adults. Future research should focus on understanding how weight

change over time, rather than static BMI measurements, contributes to health and mortality risks in older populations.

5.5 Low BMI: More Than a Lightweight Issue

While the obesity paradox has sparked considerable debate over the potential protective effects of higher BMI in older adults, an equally important, but often overlooked, aspect is the role of low BMI. Low BMI, mainly when resulting from unintentional weight loss, not only helps explain the obesity paradox but also significantly impacts health outcomes, with earlier research linking unintentional weight loss with increased mortality risk.[170–175]

In **Studies II** and **III**, low BMI was associated with higher functional, physiological, and epigenetic aging. Specifically, **Study III** highlighted the dangers of low BMI in the context of EAA and mortality. Here, low BMI was associated with higher EAA and mortality risk, although the direct effects of low BMI on survival were more pronounced than those mediated through EAA. This suggests that while accelerated biological aging may contribute to the relationship between low BMI and mortality, the more immediate concern with low BMI may be the underlying health conditions driving low BMI, such as unintentional weight loss, rather than acceleration in biological aging. Therefore, lower BMI may be a marker of vulnerability rather than resilience, often signaling declining health rather than improved well-being among older people.[13]

Study IV further supported the findings with longitudinal data, demonstrating that increased functional and physiological aging led to steeper declines in BMI in later life. As biological aging advanced, BMI declined more rapidly, reinforcing that low BMI as a clear marker of advanced aging and deteriorating health. Therefore, while low BMI may initially seem counterintuitive as a risk factor for mortality, it becomes clearer when considering the potential drivers of low BMI, such as unintentional weight loss as a result of underlying acute and chronic diseases.[170, 173]

Given the more substantial direct effects of low BMI on mortality observed in **Study III** and the evidence from **Study IV** that both functional and physiological aging contribute to BMI decline, could unintentional weight loss be a consequence of accelerated biological aging? Physiological changes associated with advanced aging — such as reduced taste and smell, slower gastric emptying, earlier satiety, and metabolic shifts that accelerate lean body mass loss — can lead to

unintentional weight loss.[161, 170, 173] These changes, especially diminished taste and smell, delayed gastric emptying, and earlier satiety, are vital indicators of appetite reduction, likely caused by age-related dysregulation of appetite control.[176] Additionally, side effects of medications, including changes in taste or smell, oral dryness, and constipation, can further reduce appetite in older adults.[176] While these physiological changes support the hypothesis that low BMI from unintentional weight loss may result from accelerated biological aging, the exact mechanisms remain unclear.

Studies II to **IV** demonstrate that lower BMI, particularly in older adults, is not simply the inverse of high BMI regarding health risk. Instead, it highlights a distinct set of risks potentially involving unintentional weight loss. The more pronounced direct effects of low BMI on survival in **Study III** also hint at the possibility of different mechanisms linking low BMI to survival compared to high BMI. Taken together, these findings remind us that weight loss in older adults often serves as a warning sign of underlying health deterioration rather than improved health. Future research and clinical interventions should prioritize understanding the causes and consequences of unintentional weight loss across the BMI spectrum in older individuals, addressing their specific needs to better manage their health risks.

5.6 High BMI: The Weighty Issue

This thesis is driven by the urgent need to understand the connection between obesity and aging, prompted by two significant global trends: the growing prevalence of obesity and the aging population. With these trends comes a critical question: does obesity accelerate aging, thereby contributing to worse health outcomes in older adults? Overlapping biological mechanisms, including chronic inflammation, oxidative stress, and cellular senescence, support the hypothesis that obesity accelerates aging.[117, 118] These mechanisms provide a glimpse into how high BMI may hasten the aging process. Throughout this thesis, the relationship between high BMI and aging was explored in multiple contexts, from the role of biological aging to its direct impact on biological aging markers and life expectancy.

In **Study II** and **Study III**, high BMI was associated with higher biological aging at the functional, physiological, and cellular levels. This supports the broader hypothesis that obesity drives aging,[117, 118] consistent with recent findings showing

hallmarks in cellular aging — such as mitochondrial dysfunction, epigenetic changes, deregulated nutrient sensing, and cellular senescence — in individuals with higher BMI contribute to the development of age-related diseases.[177] Notably, biological aging was a mediator between high BMI and mortality in **Study III**. This finding aligns with the hypothesis that obesity doesn't just directly influence health through metabolic complications but actively accelerates aging processes, which in turn contribute to earlier mortality.[117, 118]

In **Study IV**, high BMI drove higher physiological but not functional aging. According to the hierarchical model of aging metrics proposed by Ferrucci and colleagues,[82] aging progresses from molecular and cellular levels to phenotypic and functional stages. FI encompasses deficits related to functional abilities and age-related signs and symptoms, capturing functional and physiological aging aspects. In contrast, FAI focuses solely on functional aging. Interestingly, **Study II** also found the modifying effects of metabolic health in relation to physiologic aging, not functional aging, suggesting that these aging metrics may be influenced differently by various factors. Based on the hierarchical aging model, BMI's impact on biological aging might appear earlier in the FI than in the FAI. The functional aspects measured by the FAI may manifest later in the aging process. This could explain why the associations between BMI and these two indices differ in direction within **Study IV**.

Moreover, common pathophysiological changes observed in both obesity and aging, such as heightened systemic inflammation, mitochondrial dysfunction, and increased cellular senescence,[117, 118] are more closely associated with molecular and cellular aging metrics. Therefore, it's plausible that higher BMI primarily affects the earlier stages of aging at the cellular and physiological level, with less direct influence on later functional stages.

In **Study IV**, the bidirectional relationship between BMI and physiological aging highlighted the cycle of obesity and aging: high BMI contributes to accelerated biological aging, and as biological aging increases, BMI tends to decline. This dynamics underscores the complex relationship between obesity and aging, where weight loss in late life, often driven by advanced aging, complicates the simple narrative of BMI as a risk factor.

The findings from **Study II**, **Study III,** and **Study IV** substantiate the hypothesis that obesity accelerates biological aging. Whether through epigenetic, physiological, or

functional aging markers, high BMI is a significant driver of aging, contributing to worse health outcomes over time. These studies highlight the importance of addressing obesity as a metabolic issue and a central factor in the aging process. As global obesity rates continue to rise alongside an aging population, understanding how high BMI accelerates aging will continue to be crucial for developing interventions that target these biological pathways, ultimately promoting healthier aging trajectories.

5.7 Strengths and Limitations

5.7.1 Strengths

A major strength of this thesis is the use of large, population-based datasets from multiple prospective cohorts, which enabled robust and comprehensive analyses. **Studies I, II,** and **IV** utilized data from various sub-studies within the STR, including GENDER, OCTO-Twin, SATSA, and TwinGene, while **Study III** relied on data from the US Health and Retirement Study. These datasets provided a wide range of variables such as BMI, metabolic health indicators, biological aging measures, and potential confounders, allowing for a thorough exploration of the relationships under study.

Objective measurements of key variables across these studies further bolstered internal validity by minimizing biases inherent in self-reported data. For instance, height and weight were measured by trained professionals in **Studies I, II,** and **IV**, ensuring consistency and accuracy in BMI assessment. Metabolic health was assessed primarily through venous blood biomarkers, improving the reliability of health measurements. Likewise, biological aging measures, including EAA from blood samples and the functional FAI from standardized functional assessments, were objectively collected, contributing to the robustness of the findings. Although the FI relied on self-reported data, it remains a validated measure of functional and physiological change in older adults, supporting the reliability of the thesis' conclusions.

An additional strength lies in the use of multiple complementary measures of biological aging, including FAI, FI, and EAA markers. This multi-dimensional approach provides a more holistic view of aging, allowing for a nuanced examination of how BMI and metabolic health interact with different aspects of

the aging process and mortality, yielding rich insights into these complex relationships.

Furthermore, this thesis was able to analyze age-specific effects due to the inclusion of participants with a wide age range. This broad age spectrum enabled us to identify age-related patterns and differences in these relationships, providing insights into how the impact of BMI and metabolic health on biological aging may vary at different stages of midlife to late life. Lastly, the extended follow-up periods further enhanced this strength by allowing the observation of long-term trends and changes over time. This longitudinal perspective is crucial for understanding the progression of biological aging and its association with BMI and metabolic health, thereby enriching the overall findings of the thesis.

5.7.2 Limitations

A common limitation across studies is using BMI as a proxy for adiposity. While widely accepted, BMI's inability to differentiate between fat and lean mass or account for fat distribution can introduce measurement error, potentially leading to misclassification and weakening the findings' internal validity.[178] As a result, the relationships between BMI and aging may be distorted, making it more difficult to draw accurate conclusions about how body composition influences aging and health outcomes.[178]

Another challenge was classifying metabolic health status, compounded by the lack of consensus on what constitutes metabolic health. In several sub-studies, including those from the STR and HRS, the use of non-fasting glucose and lipid levels for defining hyperglycemia and hypertriglyceridemia introduced the potential for misclassification. Although only a few samples were non-fasting, and adjusted thresholds were applied, this issue remains relevant. In the OCTO-Twin cohort, the absence of triglyceride measurements meant hypertriglyceridemia was inferred through self-reported use of lipid-lowering medications, likely underestimating its prevalence. Similarly, in the HRS, hypertension classification was based solely on self-reported diagnoses, as measured blood pressure data was unavailable, while in STR, we did not rely on self-reported hypertension nor the use of anti-hypertensive, raising concerns about accuracy. Additionally, lipid-lowering medication was used to define hypertriglyceridemia and low HDL in the STR but not in the HRS. Despite these discrepancies, using MHS as a composite measure across sub-studies may have provided a more cohesive approach.

Sensitivity analyses with alternative MHS definitions were conducted across all studies to address these concerns.

Survival bias is a challenge in studies involving older populations. In particular, cohorts such as OCTO-Twin and GENDER had higher baseline ages than SATSA. This raises the possibility that healthier individuals were more likely to survive into older age and be recruited into the study, potentially skewing the results. While adjustments were made to mitigate this bias, it remains a limitation that could affect the accuracy of the findings.

Finally, the generalizability of the results is limited since the data primarily represent older adults from higher-income countries, specifically Sweden and the US. This limits the applicability of the findings to populations in low- and middle-income countries, where socioeconomic, environmental, and healthcare factors may influence the relationships between BMI, metabolic health, and aging. Moreover, the cohorts lack ethnic diversity, further constraining the generalizability of the results. Future research should aim to include more diverse populations from a broader range of socioeconomic and cultural contexts to explore potential variations in these associations.

6 Conclusions

The relationships between BMI, metabolic health, and biological aging are intricate and multifaceted. This thesis sought to untangle these connections, providing valuable insights into how they may collectively shape survival and late-life health outcomes.

Study I demonstrated that metabolic health status is a critical determinant of the BMI-mortality association. While being metabolically unhealthy with obesity in midlife, and regardless of BMI category, in late life was strongly associated with increased mortality risk, metabolic health appears to modify the impact of BMI. Specifically, individuals who were metabolically healthy overweight or metabolically healthy obesity in mid and late life were not associated with an increased mortality risk, suggesting that assessing BMI alone is insufficient. Instead, a nuanced evaluation considering both BMI and metabolic health status offers a more accurate assessment of an individual's mortality risk.

Study II reinforced the hypothesis that obesity and metabolically unhealthy status accelerate functional and physiological aging. Interestingly, both low and high BMI were associated with advanced functional and physiological aging, highlighting the importance of considering low and high BMI as potential indicators of ill health. This study suggested that integrating metabolic health and BMI provides a more comprehensive picture of overall health status, particularly in older individuals, since both high and low BMI are indicators of increased functional and physiological aging.

Study III reinforced the nonlinear association between BMI and biological aging, as seen in Study II, this time highlighting the nonlinear relationship between BMI, epigenetic aging, and risk of all-cause mortality. While both low and high BMI levels were associated with increased epigenetic aging and decreased survival time, the mediating effects of epigenetic aging were stronger in those with high BMI. In line with **Study II**, these findings support the hypothesis that obesity accelerates aging, thereby increasing mortality risk. In contrast, low BMI was associated with stronger direct effects on survival, potentially due to factors such as unintentional weight loss linked to pre-existing health conditions rather than through aging mechanisms.

Study IV highlighted the bidirectional relationship between BMI and biological aging, with higher BMI accelerating physiological aging, while higher physiological aging, in turn, contributed to steeper BMI decline. A unidirectional relationship was observed between functional aging and BMI, where higher functional aging accelerated BMI decline but not vice versa. The consistent role of biological aging as a driver of steeper BMI decline complements **Study III's** findings of weaker mediation through epigenetic aging and stronger direct effects in the low BMI and survival relationship. These findings underscore the complexity of the BMI-aging relationship, indicating that while obesity accelerates aging at the physiological level, unintentional weight loss may signal advanced functional aging. Managing obesity and unintentional weight loss, alongside preserving physical and functional capacity, emerges as a critical strategy for promoting healthy aging from midlife to late life.

This thesis provides insights into the complex entanglement between BMI, metabolic health, chronological age, and biological aging, demonstrating their joint influence on all-cause mortality. By emphasizing the intricacies of these relationships, the findings underscore the need for a more personalized approach to managing body weight and metabolic health across the aging process. Ultimately, maintaining metabolic health may be as crucial as managing body weight in promoting healthy aging and extending longevity, paving the way for more targeted interventions and future research to improve late-life health outcomes.

7 Epilogue

7.1 So What?

By now, if you've made it through my thesis, you might be asking yourself, "So what's the big takeaway here?" And that's a fair question. The studies all point to one overarching message: whether it's high BMI, low BMI, or metabolic health issues, they all affect your health in late life. No shocker, right? There's already plenty of evidence out there showing that high BMI from a young age is tied to chronic diseases and higher mortality rates.

But here's when things get interesting. If we are convinced that high BMI is already an issue in childhood and early adulthood and should be tackled earlier in life, then trying to fix it in older adults may be like trying to turn a ship that has already set sail. It's probably too late? Well, I don't have the exact findings to back this up, but we do have some hints. In Study I, we found that in midlife, only MUO was a significant mortality risk; neither MHO, MHOw, nor MUN did. Then, in Study II, we saw that high BMI was linked to higher physiological aging before age 65, but this relationship weakened after age 80. What's clear in my studies, however, is that low BMI in older adults is linked to faster biological aging and higher mortality risk. And guess what? A late-life drop in BMI is a major clinical red flag - and as Study IV suggests, it may be driven by advanced physiological and functional aging.

Perhaps it's time to rethink how we assess BMI in older adults. It's not just about checking their weight — it's about asking the right questions. What was the weight last year, two years ago, five years ago? Has there been unintentional weight loss? Could this be pointing to something more serious? The connection between low BMI and worse outcomes in this thesis should raise some eyebrows.

And let's not forget metabolic health. This isn't just a problem for those with high BMI. The data suggests that metabolic health influences aging and mortality across the board, regardless of BMI. So, perhaps having metabolic health check-ups is a good idea? And if so, that should be for everyone, not just those who appear to have overweight. For those older adults with obesity, it's even more critical to look beyond the surface. We need to assess their metabolic health, maybe factor in physical activity

and functional levels, and watch out for sarcopenic obesity — a combo of low muscle mass and excess body fat that spells trouble and a research gap.

To sum it up, we may need to take a broader, more holistic approach to BMI and metabolic health in older populations. This may mean acting earlier, taking a more comprehensive look at health, and not waiting until the later years when the damage may have already been done.

7.2 Beyond The Tip of the Iceberg

By now, perhaps it's clear to you, it's pretty clear to me — that my thesis is merely scraping the tip of an iceberg — a minuscule, visible part of a much larger and more complex field of knowledge. While this thesis offered some insights into the relationship between BMI, metabolic health, and aging, it also revealed the vast unknown beneath the surface, waiting to be explored.

One thing that's been bugging me throughout this process is the question of what really defines metabolic health. It feels like I have been just dancing around it without fully understanding. Sure, there has already been research, like GWAS, that has started to separate metabolic health from adiposity, but the next step is probably to take those findings and run with them.[166, 167] With the incredible advancements in data analytics over the past decade (my first ever Bachelor's thesis was on a floppy disk to give a sense of how far we've come), we now have the tools to dig deeper and faster — by combining multi-omics approaches to really figure out what it means to be metabolically healthy. And how about the exciting field of precision medicine, where we can start using personalized data to refine our understanding of metabolic health and how we can manage it? Plus, it's not just about the flashy new tools like machine learning or neural networks; it's also about using solid research methods, such as triangulation of evidence, to ensure we're getting a full picture and robust findings.

Also, one area I think deserves attention, which is something I couldn't fully delve into, although it has been part of the plan (I know, it's a shame), is considering the impact of cohort effects. Growing old in the 1970s is not going to be the same as growing old today. A lot has changed — not just the climate, the health policies, prescriptions

practices, diet, and lifestyles — they all have changed. What's their impact on the connection between BMI-metabolic health and aging?

And let's not forget my thesis does have a narrow focus on high-income countries. I am likely missing a huge part of the picture by not expanding these studies to low- and middle-income countries. How BMI, metabolic health, and aging connect can vary based on environmental, cultural, and socio-economic factors. We won't have a complete, globally relevant understanding of these issues until we widen the scope of our research.

Well, I can go on. But I hope this thesis has sparked your curiosity and inspired you to explore this iceberg's deeper, hidden parts because there's still so much left to uncover. Thank you so much for letting me go on this rant — it has been a journey.

8 Acknowledgments

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How many can say their supervisor packed a studio full of their personal items, carefully placed them in luggage, and shipped them across Sweden from Jönköping to Luleå? Well, I am the lucky one who has a supervisor like that. **Anna**, thank you so much for accepting me as your PhD student and for your immense kindness. Beyond that, I've learned so much from your insights and experiences in obesity and gerontology, which you share so generously.

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May everyone who is sick
Be swiftly healed,
And may every disease that affects living beings
Be permanently eradicated.

— Chapter 10, Verse 21, *Guide to the Bodhisattva's Way of Life*
by **Shantideva**

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