Healthy ageing in older adults: an exploration of interactions between lifestyle, immune, metabolic, and gut microbial health

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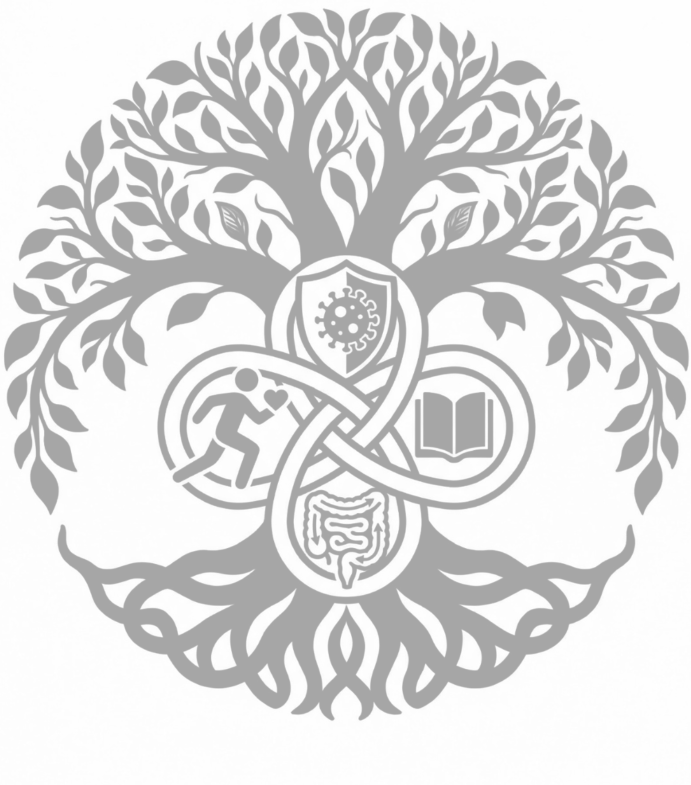

CHAPTER 1

Introduction

1. Ageing and healthspan: A growing global challenge

Across the globe, populations are undergoing a demographic transition, with a steadily growing proportion of older adults (1). Advances in medicine, public health, and living conditions have driven an unprecedented increase in lifespan, which has risen globally from around 31 years in 1900 to over 73 years today, and now often exceeds 80 years in high-income countries (2). However, the years lived free of disease and disability -healthspan- lag by 15–20 years, creating a substantial gap between living longer and living healthier (3). Evidence suggests that chronic conditions and multimorbidity are being diagnosed at younger ages, further widening this gap, although the timing of onset may vary across populations (4). As a result, the burden on healthcare systems is rising, with increasing demands for resources, care, and support for an ageing population, placing strain on both providers and system sustainability (5,6). To address this challenge, preventive strategies targeting still healthy individuals at increased risk of age-related disease are critical. Extending healthspan in this group can delay the onset of disease and reduce the future burden on healthcare systems. Therefore, prioritizing the development of evidence-based interventions in healthy older adults to extend healthspan and promote healthy ageing is vital.

2. Foundations of healthy ageing: Definitions, influencing factors, biomarkers, and targeted interventions

Healthy ageing involves maintaining functional ability and well-being in later life, allowing individuals to live fulfilling lives despite the natural physiological and psychological changes of ageing (7). Many older adults (here defined as aged 55 and older) may be clinically disease-free, yet already display early physiological vulnerabilities in key systems such as muscle, immune-metabolic function, and gut health, which indicate an increased risk of future functional decline (8–10). These vulnerabilities can be detected through biomarkers, which provide measurable indicators of biological processes in the body (11). Older adults with such early, subclinical changes represent an important target group for preventive strategies aimed at preserving health and extending healthspan (12,13). This highlights the concept of targeted intervention, which refers to designing and applying lifestyle or nutritional strategies to subgroups of individuals who share specific biological or physiological characteristics, rather than treating all older adults as a homogeneous group (14). Within research on healthy ageing multiple approaches can be distinguished, three of which are relevant to this thesis: (i) identifying modifiable lifestyle factors that can be targeted to reduce disease risk and extend healthspan, (ii) developing biomarkers to detect vulnerability and disease risk in otherwise healthy older adults, and (iii) designing effective interventions to lower disease risk, strengthen physical functioning, and improve well-being..

Modifiable lifestyle factors such as nutrition, physical activity, and sleep are key determinants of healthy ageing (15,16). While smoking remains one of the most influential lifestyle factors, it is difficult to modify, making diet, exercise, and sleep more practical and ethical targets for intervention (17–19). Adequate intake of dietary protein, fibre, and vitamin

D is critical for preserving muscle mass and shaping gut microbial metabolism, both of which are central to healthy ageing (20–22). Physical activity, particularly resistance and aerobic exercise, improves muscle function, enhances insulin sensitivity, and reduces systemic inflammation (23–25). Sleep quality and duration regulate immune function, metabolic balance, and the gut–brain axis, all of which play vital roles in maintaining health in older age (26–29). Together, lifestyle choices such as a high-protein diet, moderate-to-vigorous physical activity, and 7 to 9 hours of quality sleep represent feasible and effective targets to support healthy ageing and reduce disease risk.

Quantitative biomarkers enable the detection of early physiological changes that preceded decline of functional and mental capacity and clinical disease, providing insight into biological ageing and overall health status (11,30). Measures of body composition and muscle-related markers such as lean mass, grip strength, chair stands, and gait speed reflect functional capacity and frailty risk (11,31–33). Inflammatory biomarkers (e.g., CRP, IL-6) and metabolic indicators (e.g., fasting glucose, insulin resistance) capture systemic processes of ageing (34,35). The gut microbiome, particularly bacterial composition and diversity, has emerged as another important biomarker, with specific bacterial profiles linked to immune regulation, metabolic balance, and vulnerability to disease (36–38). Novel molecular biomarkers of ageing were generated based on -omics data (i.e. metabolomics, proteomics) in large cohort studies. These include composite measures that integrate metabolomic data into a single score of global health, disease risk, and resilience (39,40). One example of such a composite score, relevant here, is MetaboHealth generated to predict mortality at all ages in large prospective studies. The score indicates the risk of cognitive decline, hand grip strength and frailty and contains blood measures of chronic inflammation, lipids and lipid transport, glucose, albumin and amino acids, In this thesis we consider MetaboHealth as an affordable, scalable estimator of global, immune-metabolic health in older adults. Together, biomarkers of muscle function, systemic inflammation, metabolic health, gut bacterial composition, and integrated scores like MetaboHealth offer a framework for identifying older community dwelling adults at increased risk of developing age-related decline of functional capacities and disease and who may benefit most from targeted interventions.

Observational studies into ageing can reveal associations of lifestyle, behavioral and environmental risk factors on health, but can generally not establish causality. To address the non-genetic factors vital to healthy ageing,intervention studies with pre- and post-intervention measures are required. The Comprehensive Assessment of Long-term Effects of Reducing Intake of Energy (CALERIE) study demonstrated that sustained caloric restriction (25% over 24 months) reduced insulin resistance and improved biomarkers such as C-reactive protein and insulin sensitivity (41). Similarly, the Growing Old TOgether (GOTO) study combined a 25% reduction in energy intake with a 25% increase in physical activity in older adults, showing improvements in metabolic health, particularly in men, and highlighting the importance of physical activity intensity (42). The PERSonalized Glucose Optimization Through Nutritional Intervention (PERSON) study applied a dietary intervention tailored to metabolic phenotypes (muscle vs. liver insulin resistance), resulting in phenotype-specific improvements in insulin resistance and metabolism (43,44). Collectively, these studies indicate that reducing energy intake, improving diet quality, and increasing physical activity can improve health in older adults. Such targeted interventions enhance metabolic, muscle, and insulin resistance outcomes, with measurable effects on biomarkers of healthy ageing.

3. Towards understanding how combined lifestyle interventions shape muscle, immune-metabolic, and gut health to optimize targeted strategies for healthy aging

Lifestyle interventions that combine targeted nutrition and structured exercise are particularly promising for older adults, as they can promote healthy ageing, reduce chronic disease risk, and counteract declines in muscle strength and immune-metabolic health (19,45,46). Resistance training consistently increases muscle mass and strength, improves insulin resistance, and reduces the risk of functional decline in older adults (47,48). Similarly, high-protein diets support muscle protein synthesis, preserve lean body mass, and improve satiety, while prebiotic fibers modulate gut microbiota, producing metabolites that may benefit immune and metabolic regulation (48–50). Although these strategies are individually beneficial, their combined effects may exceed the sum of their parts, potentially producing synergistic improvements in muscle health, metabolic resilience, and gut function. However, evidence is limited, and the mechanisms underlying these potential benefits remain poorly defined in at-risk populations. It remains unclear how combining resistance training with a high-protein, prebiotic-enriched diet reduces disease risk and enhances muscle strength, immune-metabolic function, and gut health across vulnerable groups in both sexes.

Protein intake, resistance exercise, and sleep each influence muscle mass and strength through distinct mechanisms, including muscle protein synthesis, neuromuscular adaptation, anabolic hormone regulation, and recovery (51–53). These factors are interconnected. For example, the effectiveness of exercise on muscle may depend on protein intake, while insufficient sleep can impair anabolic signaling and recovery and reduce training benefits (54–57). Despite this, most interventions target only a single factor, limiting understanding of how diet, exercise, and sleep may reinforce or counteract one another. Individual variability in intervention responses shaped by age, sex, metabolic health, and baseline muscle status further complicates the picture (58,59). Gaining insight into the complex interplay between these modifiable lifestyle factors is essential for designing effective targeted strategies to preserve muscle mass and strength and promote healthy ageing. There is still a limited understanding of how diet, exercise, and sleep interact and independently affect muscle outcomes.

The gut microbiome is increasingly recognized as a potential biomarker of health and is clearly influenced by diet (60). However, it remains poorly understood whether other modifiable lifestyle factors, such as physical activity and sleep, shape gut bacterial species and microbial pathways in ways that reflect healthy ageing (46,61,62). Physical activity affects multiple aspects of metabolism and muscle health, yet evidence is limited on how different types and intensities of activity alter gut bacterial species and microbial pathways, especially when accounting for confounding factors such as alcohol use and diet. Sleep quality, duration, and efficiency also change with age and are linked to metabolic and muscle health outcomes, but it is unclear how these sleep parameters influence gut microbiome composition or interact with physical activity to affect muscle and overall health (63). Investigating the gut microbiome as a biomarker for healthy ageing while integrating both physical activity and sleep may reveal whether differences in bacterial composition reflect lifestyle behaviors and health status. Despite these insights, there is still limited evidence on how diet, exercise, and sleep jointly influence gut bacterial species and microbial pathways, which limits the ability to use the gut microbiome as a marker of healthy ageing and to guide lifestyle interventions.

Intervention studies such as PERSON, GOTO, and CALERIE demonstrate that precision interventions targeting metabolic or activity phenotypes can improve metabolic outcomes in older adults (41–43). However, responses vary substantially between individuals, highlighting gaps in understanding the factors that drive this variability. Identifying these sources of variation is critical for optimizing precision interventions and maximizing their benefits. Biomarkers that capture inter-individual differences could help explain why some people respond better than others and guide intervention strategies (11,30). MetaboHealth, a metabolomics-based score, is a promising candidate for this purpose because it effectively detects subtle differences in individuals' metabolic profiles, making it a potentially valuable tool for tailoring interventions (39). Despite its potential, it remains unknown whether biomarkers such as MetaboHealth can explain variability in intervention responses and support the development of more effective precision strategies to promote healthy ageing.

4. Aim and outline of the thesis

The main aim of this thesis is to assess how different modifiable factors of healthy ageing act on improvement of muscle and immune-metabolic health in ageing individuals. While a novel lifestyle intervention was designed and conducted, we aimed to disentangle the influence on health of several correlated lifestyle factors and differences in baseline immune-metabolic health that may contribute to individual variability in intervention effects. We aimed to synthesize this knowledge from observational and interventional studies on muscle, gut microbiome, and immune-metabolic health in older adults. The goal is to optimize targeted strategies for reducing disease risk and extending healthspan by lifestyle interventions.

The original plan for this thesis was based on a novel intervention in older adults, the VOILA intervention study. VOILA is a combined program incorporating resistance training, high-protein nutrition, and prebiotic-enriched nutrition in three groups of vulnerable 60+ older adults. The primary endpoints to measure the intervention effect included muscle strength, immune-metabolic function, and gut health. Secondary analyses would include the identification of factors that drive individual variability in response. However, the study was disrupted by delays, including legal restrictions that prevented starting until COVID measures were relaxed. Therefore, the design of VOILA is part of this thesis but the results are not, since the intervention was completed only at the end of the PhD trajectory. Since lifestyle interventions, especially in older adults usually display a large heterogeneity in outcomes, we were interested in lifestyle factors typical variable at baseline. Therefore, while running the multicenter VOILA study we turned to large observational cohort data repository such as UK Biobank and Lifelines, which offer the statistical power to disentangle the separate influence of several correlated lifestyle factors on health outcomes that smaller trials may miss (64,65). As illustrated in Figure 1, lifestyle factors such as exercise, diet, and sleep interact closely with gut health, immune-metabolic health, and muscle health, which together influence the ageing process. This figure also highlights how the thesis chapters are organized around these interconnected domains.

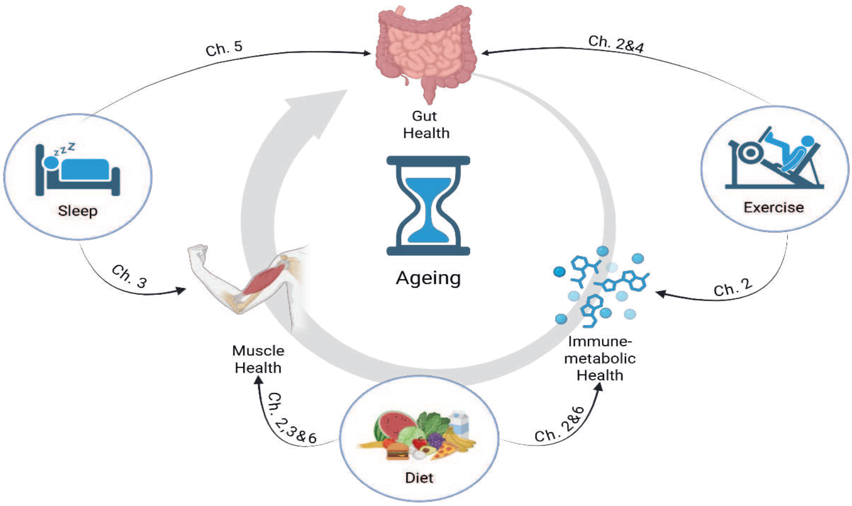

FIGURE 1. Exploring the interactions between lifestyle including exercise, diet, and sleep with gut health, immune-metabolic health, and muscle health. Created with BioRender.com. Ch: Chapter.

In Chapter 2, we designed the VOILA lifestyle intervention for older adults, combining supervised resistance training with nutritional supplementation, including proteins, prebiotic fibers, and vitamin D. Resistance training was included to enhance muscle strength and mass, while the nutritional supplementation aimed to support muscle metabolism, immune function, and gut health. Outcomes included muscle strength, body composition, immune-metabolic markers, and gut microbiome profiles, measured before and after the intervention. These datasets are currently being used to examine how the combined intervention affects muscle, immune-metabolic, and gut health, and to explore whether individual variability in response can be explained by biomarkers such as the MetaboHealth score.

In Chapter 3, we conducted a cross-sectional analysis using the UK Biobank to examine how lifestyle factors, including physical activity, diet, and sleep, relate to musculoskeletal and metabolic health in younger and older adults. The UK Biobank provides extensive phenotypic, biochemical, and lifestyle data from a large, population-based sample, enabling the study of associations across diverse subgroups and the quantification of variability in health outcomes (65). This analysis focused on how nutrition, physical activity, and sleep impact fat-free mass and handgrip strength in men and women throughout the lifespan, to inform recommendations to preserve muscle health.

In Chapter 4, we performed a cross-sectional analysis in the Lifelines cohort to investigate how different intensities of physical activity are associated with gut microbiome composition and microbial pathways. Lifelines offers a large, well-characterized population with detailed measurements of physical activity, lifestyle factors, and gut microbiome profiles, allowing us to account for confounders such as diet and alcohol use (64). This analysis aimed to determine whether physical activity intensity is linked to specific microbial profiles, providing insight into the gut microbiome as a potential biomarker of healthy aging and as a mediator of lifestyle effects.

In Chapter 5, we conducted a systematic review to summarize the evidence on how sleep disturbances affect gut microbiome composition across the lifespan and to explore how these changes may contribute to age-related muscle loss. A systematic review was chosen to comprehensively synthesize both cross-sectional and experimental studies, identify consistent patterns, and highlight gaps in the literature that individual studies cannot address. This review specifically aimed to examine how sleep quality and duration influence gut microbiome composition and whether these microbial changes may help explain the link between sleep disturbances and declines in muscle health.

In Chapter 6, we analyzed data from the PERSON study, a 12-week, tissue-specific dietary intervention in which participants with muscle insulin resistance received a high-MUFA diet and those with liver insulin resistance received a low-fat high-protein diet, with metabolic and functional outcomes assessed before and after the intervention (43). The PERSON study was selected because it is a well-characterized, controlled, two-center trial with available Nightingale metabolomics data, enabling calculation of the MetaboHealth score and providing a unique opportunity to investigate targeted nutrition strategies. This analysis aimed to determine whether the MetaboHealth score can explain variability in response to the dietary intervention and help stratify individuals based on their insulin resistance type and MetaboHealth profile.

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