General Introduction

MUSCULOSKELETAL AGING: OSTEOPOROSIS, SARCOPENIA, FALLS AND FRACTURES

"Aging is inevitable. Healthy aging is not"

Aging is a natural and inevitable part of our lives. Over the past decades, the average global life expectancy has been increasing at a rapid rate due to the decrease in all-cause mortality. This has resulted in significant growth of the fraction of older people in the population, especially among those 65 years and over, including centenarians (reaching an age of 100 years).[1] In the last five decades, the number of people aged 50 and older has quadrupled to more than 1.6 billion. Accordingly, the prevalence of chronic diseases has also increased, with more than 80% of the individuals above the age of 65 suffering at least one chronic condition.[2] A substantial proportion of this disease load is attributed to musculoskeletal disorders.[3] With aging the musculoskeletal tissues function less effectively due to changes in their quantity and quality. [4] Several pathological mechanisms can explain the tissue alterations with aging such as imbalance in matrix synthesis and degradation, altered matrix composition and decline in the number of effective stem cells (**Box 1**).[4] When tissue damage accumulates and exceeds a certain threshold it becomes clinically evident and manifest across muscle, bone, cartilage, and tendons.

Box 1 | Mechanisms driving the age-related musculoskeletal tissue changes

- Decrease in the amount of tissue, usually secondary to an acquired imbalance in matrix synthesis and breakdown.
- Altered molecular composition of the matrix, particularly post-translational modification of structural proteins such as collagen and elastin.
- Accumulation of degraded molecules in the matrix.
- Reduced efficiency of functional tissue elements.
- Reduced synthetic capacity of differentiated cells.
- Decline in effective stem cell populations.
- Altered levels of circulating trophic hormones, growth factors and cytokines, or an altered ability of the cells to respond to them.
- Alterations in the loading patterns of the tissue or the tissue's response to loading.

Source: Freemont, A. & Hoyland, J. Morphology, mechanisms and pathology of musculoskeletal ageing. J. Pathol. 2007

Musculoskeletal disorders and their sequels are the most common cause of pain and physical decline in the elderly and are among the leading contributors of years lived with disability worldwide. [5] The decline in bone mass and quality i.e., **osteoporosis,** and muscle mass and strength i.e., **sarcopenia,** are highly prevalent in elderly people

and are the main focus of this thesis together with their sequels i.e., **falls and fractures**. Both falls and fractures represent a serious health issue leading to loss of confidence and independence, increased morbidity and mortality that together impose a large burden on the healthcare system, patients, their families and society in general. Thus, besides aiming to further extend the lifespan what is more relevant now is procuring to achieve a longer *healthy* lifespan. Identifying factors leading to sustainable aging free of morbidity e.g., musculoskeletal and co-morbidity is a pivotal step in the realization of the ideal of healthy aging.

"...years are being added to our lives, life is not being added to our years: the extra years are being added at the very end of our lives and are of poor quality" [6]

Guy C Brown

CONTEMPORARY DEFINITIONS OF OSTEOPOROSIS AND SARCOPENIA

Osteoporosis, derived from the Greek terms for ***bone and pore***, was coined in the mid-1830s to describe porous bones. Many definitions of osteoporosis have followed and in the early 1990s WHO issued a consensus statement which defined osteoporosis as a systemic disease characterized by ***low bone mass and microarchitectural deterioration of the bone tissue leading to increased fracture susceptibility***. From this definition, clinical cut-off points were established for bone mineral density (BMD) measured by Dual-energy X-Ray Attenuation (DXA) at the femoral neck or the lumbar spine (the most prominent skeletal sites of fracture). A diagnosis of *osteoporosis* conforms to BMD levels lower than -2.5 SD below the reference for young adults, while BMD levels between -1 and -2.5 constitute *osteopenia*. [7] This classification has been widely adopted in clinical practice and -very importantly- to define treatment indication. While DXA-based BMD provides a quantifiable assessment of fracture risk, it comes short to assess properties of bone mass distribution and microarchitecture. Hence, other assessments like Trabecular Bone Structure (TBS) [8] determined from DXA images have been developed. Further, as DXA measurements are two-dimensional and only provide a measure of areal BMD, additional methods such as peripheral Quantitative Computerized Tomography (pQCT) have evolved to provide additional information regarding bone volume and/or microarchitecture for which clinically relevant thresholds are yet to be defined (**Figure 1**).

Sarcopenia, derived from the Greek phrase ***poverty of the flesh***, was defined for the first time in the late 1980s as an age-associated loss of lean mass. [9] However, since with aging two additional aspects of the muscle are changing i.e. strength and

performance, the definition of sarcopenia has been evolving over the years supported by collaborative efforts from several research groups around the world. [10,11,12,13] Currently, all distinct consensus definitions characterize sarcopenia by the presence of both ***low muscle mass and reduced muscle function (strength or performance)***. There is a wide variety of diagnostic tests and tools available to determine sarcopenia (**Figure 1**). However, an agreement on the "*golden standard*" tool and cut-off points to define this condition, is still lacking. As consequence, the comparison of observational studies is challenging and the early diagnosis and intervention of sarcopenia can also be hampered. To facilitate the clinical diagnosis of sarcopenia, the European Working Group on Sarcopenia in Older People (EWGSOP) has provided recommendations for early detection of sarcopenia in clinical practice. [14] The biggest milestone was the recognition of sarcopenia as a clinical condition in 2016 (ICD-10-CM M62.84). [15] Sarcopenia is clinically relevant syndrome as it is associated with variety of adverse health outcomes, such as disability, falls and increased mortality. Therefore, it is important to raise the awareness about sarcopenia among the medical professionals and the general population.

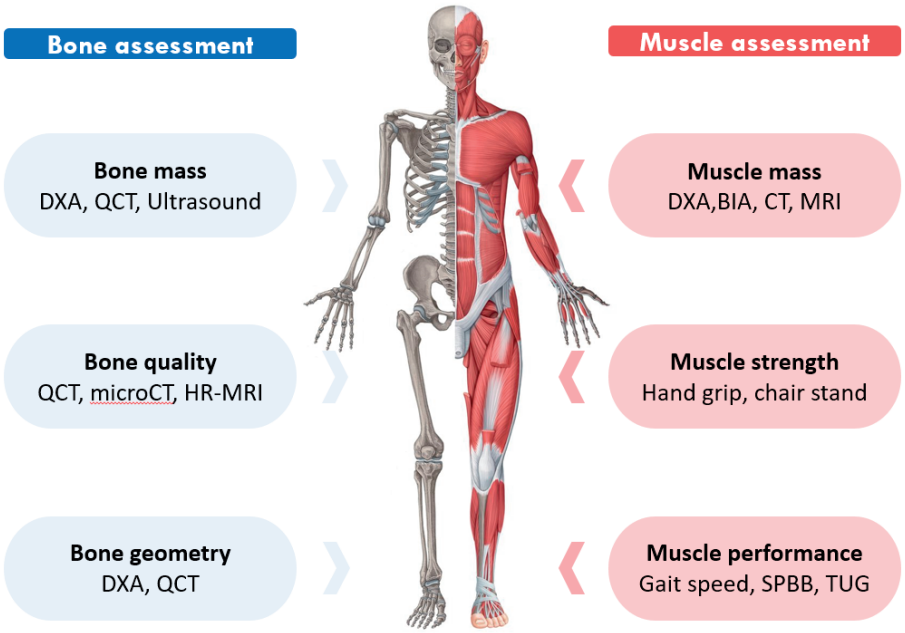

Figure 1 | Non-invasive techniques for assessing bone and muscle mass and quality.
DXA= dual-energy X-ray absorptiometry; BIA= bioelectrical impedance analysis; QCT= quantitative computed tomography; MRI= magnetic resonance imaging; HR= hight resolution; SPBB= short physical performance battery; TUG= timed up and go.

CONSEQUENCES OF OSTEOPOROSIS AND SARCOPENIA: FRACTURES AND FALLS

Each year, almost a third of all people aged 65 and over fall at least once and there are an estimated 8.9 million new fragility fractures worldwide. [16] Fragility fractures are fractures that result from mechanical forces that would not ordinarily result in fracture, i.e., low energy trauma.[17] They are highly prevalent in individuals over 50 where approximately one in two women and one in four men will experience at least one major osteoporotic fracture (hip, wrist, humerus or clinical vertebral fracture). The age-related decline in BMD is the strongest non-modifiable risk factor for fracture. Besides age many other modifiable risk factors can lead to low BMD and significantly increase the fracture risk; thus, early identification of people at higher risk of developing osteoporosis, and subsequent intervention, can have tremendous effects in fracture prevention. Muscle mass is an important determinant of BMD. It can determine the rate of both bone accrual in children [18] and bone loss in older adults. [19] Sarcopenia has been associated with increased fracture risk, however, these effects have been mainly driven by low BMD. [20,21] Nevertheless, sarcopenia can lead to fracture by pathways independent of BMD such as risk of falling. Indeed, sarcopenia is one of the many modifiable risk factors for falls [22] and the risk of falling appears to increase with the number of risk factors such as changes in vision, balance, sedentary lifestyle and medication use. [23] Falls are a relevant predictor of fracture causing almost 90% of all hip fractures [24] and within the first six months after hip fracture, up to half of the people fall again. [25] Prevention strategies focused on improving muscle and bone health will substantially decrease the falls and fracture incidence and reduce the associated physical and economic burden in the following years.

DETERMINANTS OF OSTEOPOROSIS AND SARCOPENIA: WHEN NURTURE AND NATURE COLLIDE

"The problem, of course, with the idea of nature versus nurture was that it posed a choice between determinisms." *James S.A. Corey*

Variety of **genetic, epigenetic, mechanical, biochemical, and lifestyle factors** determine the growth, accrual, maintenance and, loss of bone and or muscle mass through the life-course. **Common risk factors** for osteoporosis and sarcopenia include age, sex, sedentary lifestyle, poor diet, smoking, alcohol consumption, corticosteroids, low vitamin D, co-pathology [26,27] (**Figure 2**). In addition to these risk factors, osteoporosis and sarcopenia are interconnected with the constant mechanical and biochemical cross-talk within the bone-muscle unit [28]. For instance, bone properties are regulated

by **mechanical forces** at different levels, as demonstrated by the various developmental and functional aberrations that arise in the absence of muscle stimuli or loadings [29]. In this fashion, the loss of muscle mass and strength in older adults will have impact on the rate of bone loss as well. Furthermore, a variety of anabolic or catabolic molecules released by skeletal muscle (e.g. myokines, IFG1, FGF2) [30] or by bone (e.g. osteocalcin, sclerostin, IGF-1) [31,32] may have beneficial or detrimental effects on each other (**Figure 2**).

The effects of the environment are important; however, our phenotype is also in part shaped by variation in our **genome** (DNA). Some phenotypes such as the colour of our eyes or hair are largely determined by genetics. Similarly, monogenic disorders, i.e., Mendelian diseases, are strongly influenced by variation in one single gene. On the other hand, sarcopenia and osteoporosis are complex disorders which are result of the joint effect of numerous variations across the genome and their interaction with environmental factors. The most common genetic variation is the **single nucleotide polymorphism (SNP)** defined as a variation in a single DNA base (A, C, G or T) which can be detected using existing SNP genotyping assays. With the advent of SNP genotyping microarray technology, it is now possible to genotype a proportion of SNPs

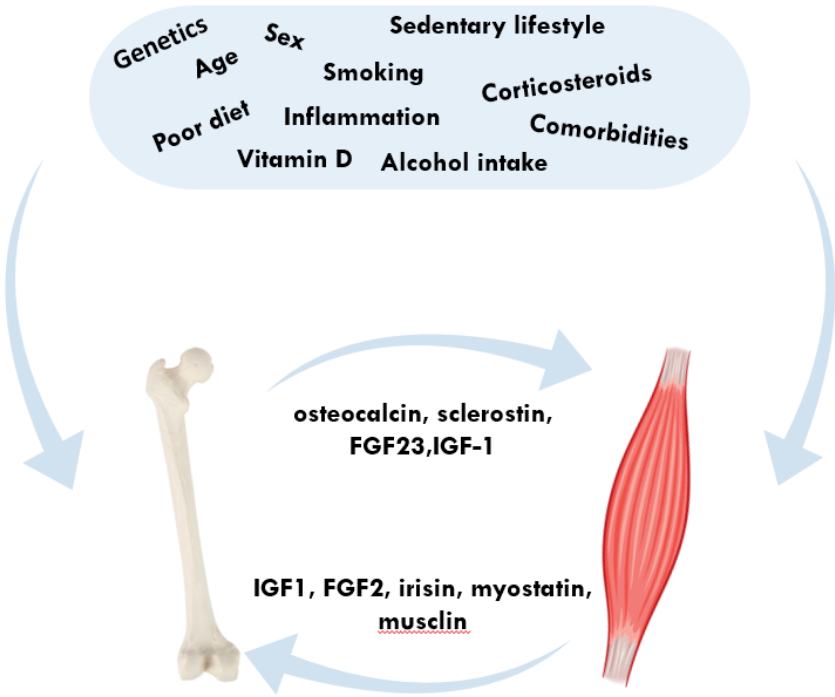

Figure 2 | Risk factors affecting both muscle and bone, and muscle-bone cross talk.
IGF= insulin growth factor; FGF= fibroblast growth factor.

(typically around 500,000), while the reminder (up to 300 000 000) are imputed using reference panels of individuals with dense genotype data such as HapMap, or the 1000 genome, HRC or TOPMed projects (among others). Using **genome-wide association studies (GWAS),** we can analyse these measured variations i.e. SNPs in relation to specific traits or diseases within a hypothesis-free approach (i.e., comprehensive genome scans without any previous knowledge on gene function). The effect sizes of the causal genetic variants and their corresponding frequencies are the determinants of the genetic architecture of a given trait or phenotype. For common diseases and complex traits, effects per SNP are typically small arising from common to less frequent variants. Hence, the power to detect associated genetic variants is highly driven by the sample size of a study (or meta-analyses thereof). The larger the sample size the higher the power to detect novel associated variations across the genome. In order to maximize the statistical power many collaborative efforts have been established across the world which have had unprecedented success, and the musculoskeletal field has been no exception (reviewed in **Chapter 3.2.1**[33] and **Chapter 4.1**[34]). These genomic discoveries have substantially expanded our knowledge of the gene-disease relationships.

Moreover, **epigenetic regulation** of bone and muscle metabolism have been shown to also play a role. Studies have shown, for instance, that microRNAs are associated with bone and muscle homeostasis [35,36]. Similarly, (total body) muscle mass and (total body) bone mass are highly genetically correlated (r~0.40)[37]; indicating that there is overlap between genetic influences on bone and the genetic influences on muscle. Significant genetic overlap across phenotypes can be due to pleiotropy, strictly speaking, when one gene controls the expression of multiple traits. To date, several genes have been proposed to affect both bone and muscle, such as *SREBF1, GLYAT* and *METTL21C* (reviewed in **Chapter 4.1**)[37,38,39]. There are several methods to investigate pleiotropy namely classified as univariate and bi-/multivariate. The latter methods require all phenotypes to be measured on the same individual, i.e. individual level data; whereas, univariate methods are based on GWAS summary statistics data of one trait. Identifying pleiotropic genes can be beneficial for the joint treatment of both sarcopenia and osteoporosis, i.e., osteosarcopenia, which often co-occur in elderly people.

"In the real world there is no nature vs. nurture argument, only an infinitely complex and moment-by-moment interaction between genetic and environmental effects." *Gabor Mate*

SCOPE OF THIS THESIS

The **aim** of this thesis is to identify genetic and environmental risk factors of osteoporosis, sarcopenia and their sequels i.e., falls and fractures. The epidemiological studies were embedded within the **Rotterdam Study (RS)** a prospective population-based study including inhabitants of the Ommoord district in the city of Rotterdam, the Netherlands and divided into three cohorts [40]. Participants underwent a wide range of periodic examinations in a research facility in the centre of their district, repeated every 3-4 years using and complemented with home interviews. The Genetic Laboratory of the Erasmus MC preformed the genotyping of all three RS cohorts. The study is approved by the medical ethics committee and all participants have provided written informed consent to participate in the research. Genetic studies of the RS are carried out within the setting of large collaborative efforts, namely the **GEnetic Factors for OSteoporosis (GEFOS)** and **Cohorts for Heart and Healthy Research in Genomic Epidemiology (CHARGE)** consortia. These are international networks involving various prominent research groups around the world with a general objective to identify the genetic determinants of musculoskeletal and aging-related traits [41]. In addition we made use of the **UK Biobank Study**, one of the largest genetic biobanks in the world [42].

Chapter 2 addresses the determinants of osteoporosis and fracture risk. In **Chapter 2.1.1,** we estimated the incident rates and trends of site-specific non-vertebral fractures in the Rotterdam Study over 20 years of follow-up and determine the predictive value of one single BMD measurement. **Chapter 2.1.2** is an observational study which explores the effects of metabolic syndrome and its components on different aspects of bone health. **Chapter 2.1.3** examines the association between osteocalcin, adiposity and bone health in elderly people using a cross-sectional study design. **Chapter 2.2.1** is a review paper summarizing the current state and evolution of the studies on the genetics of osteoporosis and fracture risk. **Chapter 2.2.2** is a GWAS study which aimed to disentangle the genetic background of circulating osteocalcin. **Chapter 2.2.3** provides insights into the genetics of total body BMD and examines possible age-specific effects using GWAS approach while **Chapter 2.2.4** explores the genetic and clinical determinants of risk of fracture within a GWAS and MR study design (**Box 2**). **Chapter 3.2.5** is a review paper of the causal inference (Mendelian randomization) analyses that have been performed in relationship with bone. Next, **Chapter 3** focuses on sarcopenia, considered a novel geriatric syndrome, and its risk factors and consequences. In **Chapter 3.1,** we estimated the phenotypic correlations between a variety of bone and muscle parameters derived from DXA and pQCT. In **Chapter 3.2,** we estimated the overall prevalence of sarcopenia in older adults and explored the most common clinical correlates of this pathology. In **Chapter 3.3,** we conducted a systematic review and meta-analysis to estimate the overall prevalence

of sarcopenia in patients with chronic obstructive pulmonary disease whereas in **Chapter 3.4** we evaluated the relationship between body composition and sarcopenia with non-alcoholic fatty liver disease in an observational study of elderly people. **Chapter 4** examines the pleiotropic effects in the musculoskeletal system **Chapter 4.1** is a review paper on the current knowledge of the bone and muscle interactions in humans. In **Chapter 4.2,** we used a bivariate genome-wide approach to search for possible pleotropic genes affecting bone and muscle whereas in **Chapter 4.3** we explored the genetic landscape of falling risk by preforming GWAS analysis and estimated the shared heritability of falls with bone and muscle phenotypes. In **Chapter 4.4** we examined the shared genetics between osteoarthritis and BMD using a systematic overlap analysis on a genome-wide scale. Finally, **Chapter 5** discusses the findings and provides suggestions for future research.

Box 2 | Glossary of Mendelian randomisation

Mendelian randomization (MR) is a statistical technique that leverages genetic information in order to provide evidence for a causal relationship between modifiable risk factors and diseases.

Natural experiment

The MR approach uses genetic variants as instrumental variables for the risk factor of interest. Due to the random allocation of alleles during gamete formation the genetic variants are less likely to be associated with any confounders. Importantly, they cannot be affected by reverse causation. Therefore, the MR approach can provide more robust evidence of causal associations compared to the traditional observational studies.

Key assumptions

1. The genetic variants must be associated with the risk factor under investigation.
2. The genetic variants are not associated with any confounder that can bias the association between the risk factor and the outcome.
3. The genetic variants affect the disease under investigation only through the risk factor of interest.

Study design

One sample MR – when both the risk factor and outcome are measured in the study population.

Two sample MR – when the risk factor and outcome are measured in two different study populations. This methodology has been facilitated by the advent of large-scale GWAS that have led to substantial increases in the statistical power of the MR approach.

Limitations

- Heterogeneity – Presence of differences in effects estimates between the genetic variants used as instrumental variables for the risk factor under investigation that cannot be explained by sampling variation alone.
- Population stratification – Presence of differences in allele frequencies and/or disease prevalence rates between subgroups in the total study population which can confound the association between the risk factor and the disease of interest.
- Pleiotropy – When one genetic variant is associated with more than one trait which is a serious violation of the third MR assumption.
- Canalization – When the individuals' response to genetic and environmental influences is attenuated or absent as a result of the presence of so-called "buffering mechanisms" that act against the expected genetic and environmental effects.
- Weak instruments – When the genetic variants explain a small proportion of the variation of the risk factors, MR can provide biased causal estimates due to very low statistical power.

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