Is thyroid status a common denominator of age-related disease?

Vliet, N.A. van

CHAPTER 2

Viewpoint on the role of tissue maintenance in ageing: focus on biomarkers of bone, cartilage, muscle, and brain tissue maintenance

Evie van der Spoel, Nicolien A. van Vliet, Diana van Heemst

Ageing Res Rev 2019 Dec;56:100964.

ABSTRACT

Specific hallmarks are thought to underlie the ageing process and age-related functional decline. In this viewpoint, we put forward the hypothesis that disturbances in the process of tissue maintenance are an important common denominator that may lie in between specific hallmarks of ageing (i.e. damage and responses to damage) and their ultimate (patho)physiological consequences (i.e. functional decline and age-related disease). As a first step towards verifying or falsifying this hypothesis, it will be important to measure biomarkers of tissue maintenance in future studies in different study populations. The main aim of the current paper is to discuss potential biomarkers of tissue maintenance that could be used in such future studies. Among the many tissues that could have been chosen to explore our hypothesis, to keep the paper manageable, we chose to focus on a selected number of tissues, namely bone, cartilage, muscle, and the brain, which are important for mobility and cognition and affected in several common age-related diseases, including osteoporosis, osteoarthritis, sarcopenia, and neurodegenerative diseases. Furthermore, we discuss their advantages and limitations for use in (pre)clinical studies. The proposed biomarkers should be validated in future research, for example by measuring these in humans with different rates of ageing.

INTRODUCTION

The ageing population is growing, with implications for nearly all sectors of society. Therefore, improving health at old age is currently one of the central aims of biomedical research. Specific hallmarks are thought to underlie the ageing process and age-related functional decline and disease. In this viewpoint, we put forward the hypothesis (**Figure 1**) that disturbances in the process of tissue maintenance are an important common denominator that may be at the interface between specific hallmarks of ageing (i.e. damage and responses to damage) and their ultimate (patho)physiological consequences (i.e. functional decline and age-related disease). Crucial for ageing research is acquiring accurate quantifiable biological markers, i.e. biomarkers, of biological age[1]. Biological age is different from chronological age and represents the heterogeneity in physiological health between individuals of similar calendar age; someone with a younger biological age is considered to have a physiologically younger body and hence a longer remaining life expectancy compared to someone of the same calendar age but with a higher biological age. Being able to assess biological age at the level of the individual will improve clinical decision making and the definition of endpoints for intervention studies[2]. Ageing can be described as time-dependent functional decline characterized by the progressive loss of physiological integrity, which results in an increased vulnerability to disease and mortality[3]. As a result, ageing is among others associated with loss of independence and self-reliance. A study performed by Fried *et al.* showed that maintaining independence was the most important health outcome for most older persons, compared to symptom relief, pain relief, and staying alive[4]. Also from a societal perspective, prolonging the independence and self-reliance of older people is of importance. Crucial for independence are, among several other factors, mobility, requiring the proper functioning of the musculoskeletal system, and cognition, requiring the proper function of the brain. As a first step towards verifying or falsifying the hypothesis that disturbances in the process of tissue maintenance are an important common denominator that may be at the interface between specific hallmarks of ageing and functional decline and age-related disease, it will be important to measure biomarkers of tissue maintenance in future studies in different study populations. The main aim of the current paper is to discuss potential biomarkers of tissue maintenance that could be used in such future studies.

Among the many tissues that could have been chosen to explore our hypothesis, to keep the paper manageable, we chose to focus on a selected number of tissues, namely bone, cartilage, muscle, and the brain, which are important for mobility and cognition and affected in several prevalent age-related diseases, including osteoporosis, osteoarthritis, sarcopenia and neurodegenerative diseases. Osteoporosis is characterized by reduced bone mass, strength,

and microarchitecture, leading to increased fracture risk[5]. Osteoarthritis is an inflammatory process of the joints leading to damage and loss of cartilage with consequential joint pain and loss of joint mobility[6], while sarcopenia is characterized by the combination of loss of skeletal muscle mass with loss of muscle strength and/or physical performance[7,8]. Progressive degeneration of neurons in the brain leads to neurodegenerative diseases including Parkinson's, Alzheimer's, and Huntington's disease[9]. Ageing is accompanied with declines in almost all our physiological systems, including the cardiovascular, respiratory, and urogenital systems, and all of these systems are crucial for maintenance of independence into old age. However, to discuss all these systems is beyond the scope of this paper. Therefore, the focus of this paper will be on the discussion of biomarkers for bone, cartilage, skeletal muscle, and brain tissue maintenance.

Several research groups were already successful in discovering biomarkers that indicate physiological and/or (sub)clinical (loss of) function of bone, cartilage, skeletal muscle, and brain, including bone mass, grip strength, gait speed and verbal fluency[10-13]. Preferably, biomarkers would be related to an early stage of the ageing process, where effective intervention could take place. Although some previously defined biomarkers of ageing are indeed related to earlier stages of the ageing process, we propose here to identify biomarkers specifically associated with (loss of) tissue maintenance. The specific processes underlying deficits in tissue maintenance are categorized in the conceptual framework of the hallmarks of ageing[14]. Factors that are associated with the different hallmarks of ageing have been investigated as possible biomarkers of ageing. Since these biomarkers have been extensively reviewed by others, these biomarkers will only briefly be discussed in this paper. The main focus of this viewpoint is on the hypothesis that disturbances in tissue maintenance are at the interface between specific hallmarks of ageing and functional decline and age-related disease.

Tissue maintenance and repair requires the replacement of lost and/or dysfunctional cells. The correct activation, amplification, and differentiation of functional adult stem cells is critically important for maintenance of the regenerative potential of tissues. Tissue regeneration continues throughout life resulting in a constant renewal of cells in the body, in order to compensate for the continuous loss of functional cells caused by exposure to internal and external stress-factors [15]. Tissues differ in the rates at which cells are damaged and/or lost and replaced, and as a consequence in their rate of regeneration. If tissue maintenance would be perfect, tissues could be life-long regenerated. However, reductions in numbers and flaws in the activation, amplification, and/or differentiation of functional stem cells on the one hand as well as increases in numbers and flaws in the detection, repair, and/or removal of dysfunctional cells on the other hand may lead to a reduced ability to regenerate tissues.

Therefore, we hypothesize that the capability of maintenance of tissue homeostasis decreases with age, leading to loss of function and eventually death. In contrast, longevity could be the result of a prolonged ability to maintain tissue homeostasis. Factors that reflect the activity of processes related to tissue homeostasis (i.e. the ability of repair and maintenance of tissues) might be potential biomarkers of biological age, but might also be potential early markers of age-related diseases. We propose that biomarkers of tissue maintenance comprise a novel category of biomarkers of ageing, being more tissue specific than biomarkers related to hallmarks of aging while being potentially an earlier marker of decline than the biomarkers indicating loss of function. In **Figure 1**, this central hypothesis is visually presented. However, these biomarkers should be first measured in ageing research to assess their validity before these can be used in clinical studies.

Here, we give an overview of (established) biomarkers of tissue maintenance and we will discuss their advantages and limitations for use in (pre)clinical studies. As described before, we will focus on bone, cartilage, skeletal muscle, and the brain, and on the pathophysiological processes in these tissues/organs that underlie four of the main age-related diseases; osteoporosis, osteoarthritis, sarcopenia, and neurodegenerative diseases respectively. Biomarkers described in this paper do not necessarily have to be causally involved in the process of regeneration; they could also be by-products of building up tissues or waste products of breaking down tissues. We do not aim to give a comprehensive overview of all the possible biomarkers of maintenance of these tissues, but rather a starting point when searching for potential biomarkers to measure in the context of ageing and tissue maintenance. In future studies, these biomarkers of tissue maintenance could be validated, for example by measuring them in humans with different rates of ageing.

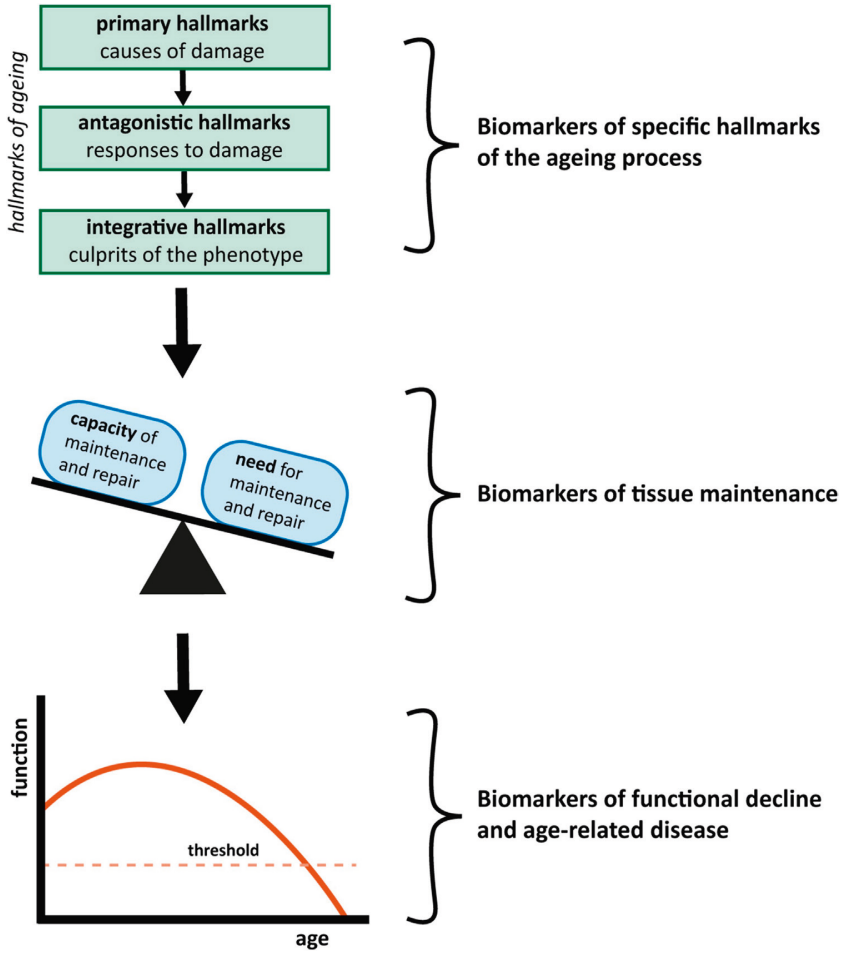

Figure 1. Hypothesis: biomarkers of tissue maintenance are at the interface between the hallmarks of ageing and age-related functional decline and disease

There is a constant balance between the capacity and need for tissue maintenance and repair during life. However, it is hypothesized that the capability of maintaining tissue homeostasis decreases with age while the need for maintenance and repair increases. Consequently, due to evolved limitations in maintenance and repair, molecular damage will accumulate gradually, and interfere with the integrity of cells and tissues, thus driving functional decline and risk of age-related diseases and death. Factors that reflect the activity of processes related to tissue maintenance could therefore represent a novel category of biomarkers of ageing; at the interface between biomarkers of specific hallmarks of ageing (i.e. damage and responses to damage) and their ultimate (patho)physiological consequences (i.e. functional decline and age-related disease). However, these biomarkers should be first measured in ageing research to assess their validity before they can be used in clinic.

Biomarkers of biological age

Biomarkers are defined by the World Health Organization as "any substance, its products, structure or process that can be measured in the body and that influences or predicts the incidence of outcome or disease" [16]. Preferably, the measurability of a biomarker is accurate and reproducible, and the biomarker has a validated association with a specific biological process or outcome, resulting in low false negatives and false positives. Intuitively, an optimal panel of biomarkers would consist of multiple biomarkers, in order to represent changes in specific organs and across different organs. Furthermore, an ideal biomarker is measured in bodily fluids that can be obtained relatively non-invasively, including blood, saliva, and urine, is assessed by a little time-consuming and low-priced assay, which is applicable for use in large-scale studies.

Biological age of an individual is challenging to measure. One of the reasons for this is that the ageing process is dependent on various external and internal factors, including genetic, environmental, and chance factors. Consequently, the human population, and especially older individuals, are a heterogeneous group. Nevertheless, several research groups were successful in discovering biomarkers that are associated with clinical features of ageing or with chronological age [10-13]. The ageing process becomes most noticeable when functions, including metabolic, cardiovascular, and/or cognitive function, decline under a certain threshold. Therefore, functional tests could serve as biomarkers of ageing. Tests of physical function that correlate well with biological age and/or mortality are hand grip strength, gait speed, chair stand test, and global Mini-Mental State Examination (MMSE) test [10, 17-20]. These markers are mostly related to a clinical feature of an older person, thus with a late phase of the ageing process. Consequently, there are fewer possibilities to intervene with the ageing process at this stage. Preferably, biomarkers of ageing are causally associated with key features of the ageing process itself.

A few years ago, Lopez-Otin *et al.* proposed to capture and categorize our currently available knowledge about the ageing process into a conceptual framework that comprises nine universal hallmarks of ageing, namely the primary hallmarks genomic instability, telomere attrition, epigenetic alterations, and loss of proteostasis, the antagonistic hallmarks deregulated nutrient sensing, mitochondrial dysfunction, and cellular senescence, and the integrative hallmarks stem cell exhaustion, and altered intercellular communication [14]. Theoretically, factors associated with one or more hallmarks of ageing could be potential causal biomarkers of ageing. In fact, numerous research groups have been working on the discovery of new and accurate biomarkers which are associated with the hallmarks of ageing [21]. The primary hallmarks of ageing reflect the accumulation of damage. Of these, diminished DNA repair

with ageing could for example be determined by measuring poly(ADP-ribose) polymerase 1 (PARP-1) expression levels, since this enzyme is one of the key players in DNA damage repair [22]. Also leukocyte telomere length is a potential biomarker of ageing, but the clinical evidence is yet inconclusive [23]. Epigenetic alterations such as DNA methylation, which involves addition or removal of methyl groups on cytosines in cytosine-phosphate-guanine (CpG) dinucleotides, can be measured using DNA methylation array technology. Several research groups are working on 'epigenetic clocks' in which these sets of CpGs are coupled with a mathematical algorithm to estimate the age of an individual. These epigenetic clocks are a promising molecular estimator of biological age [24, 25]. Another hallmark of ageing, loss of proteostasis, could be determined by the analysis of N-glycomic changes in glycoproteins, Advanced Glycation Endproducts (AGEs), or protein damage in blood [12]. Responses to damage during the ageing process are categorized as antagonistic hallmarks of ageing, including deregulated nutrient sensing. The insulin/insulin-like growth factor 1 (IGF-1) signaling pathway is important for nutrient sensing, and is one of the most evolutionarily conserved pathways across species that has been associated with ageing and longevity [26]. Furthermore, glucose tolerance and insulin sensitivity decline with age, which increases the risk for various age-related diseases. Insulin sensitivity and glucose tolerance are routinely assessed by measuring fasting glucose and insulin levels and by an oral glucose tolerance test [27]. Mitochondrial dysfunction results in the production of reactive oxygen species (ROS) which causes cellular damage [28]. However, the effects of ROS on ageing are probably dose dependent. Another hallmark of ageing is cellular senescence, which is defined as irreversible cell cycle arrest and which plays a role in preventing tumor formation. Although more research is required in this area, senescence seems best described by a combination of biomarkers of cellular senescence including β-galactosidase, elevated expression levels of p16INK4a and hypophosphorylated nuclear retinoblastoma protein, short or dysfunctional telomeres, senescence-associated heterochromatic foci (SAHFs) and γH2AX (DNA damage) foci, and senescent cells exhibit a senescence associated secretory phenotype (SASP) [29, 30]. The integrative hallmarks comprise stem cell exhaustion and altered intercellular communication. Hematopoietic stem cells (HSCs) and progenitor cells (HSPCs), which are located in the bone marrow, renew blood cells, comprising red cells, white cells, and platelets. Surface markers of HSCs and HSPCs are Lin−CD34+CD38−CD90-/+CD45RA−Flt3+CD7−CD10−, which could be potential markers to determine the level of stem cell exhaustion [31]. Besides increases in immunosenescence and inflammaging, features of altered intercellular communication comprise the decline in sex hormones with age, including estrogen and testosterone [32, 33]. All of these processes and hallmarks may ultimately result in an age-related decline in tissue maintenance. Identifying biomarkers of tissue maintenance is the central focus of the current paper.

Bone tissue

Bone is a connective tissue in which different types of cells are separated by a matrix that is composed of collagen fibres impregnated with minerals. Bone tissue is dynamic as it is continuously resorbed, renewed, and remodeled. In healthy adults, approximately 25% of trabecular bone, the internal tissue of the skeletal bone with an open cell porous network, is remodeled annually. The outer denser layer, cortical bone, remodels less frequently [32]. The whole skeleton is renewed every 7-10 years. During the bone remodeling cycle, bone is resorbed at approximately the same rate as new bone is formed. Within the basic multicellular unit, bone cells control bone formation and resorption. Bone turnover starts with resorption of old or damaged bone by osteoclasts, followed by the formation of new bone by osteoblasts, which is partly regulated by osteocytes. Osteocytes are transformed osteoblasts when surrounded by osteoid [34]. Mechanical loading is important for maintaining bone mass and studies have shown that both bone formation and resorption markers are increased after physical activity [35].

Osteoporosis

Bone resorption starts to exceed bone formation around the age of 30 years, causing gradual bone loss with ageing. This gradual bone loss could result in osteoporosis which is characterized by reduced bone mass, strength, and microarchitecture, leading to increased fracture risk. The prevalence of osteoporosis increases with age, and especially postmenopausal women have an increased risk of osteoporosis because of the reduction in estrogen. Approximately 6% of European men and 21% of European women in the age category of 50-84 years are diagnosed with osteoporosis [5]. Besides age and being postmenopausal, other risk factors for low bone mineral density (BMD) are family history of osteoporosis, cigarette smoking, low physical activity, low body mass index, low calcium intake, low vitamin D level, and use of corticosteroids [36]. Osteoporosis is primarily diagnosed by BMD of the spine and proximal femur, which is measured by dual-energy X-ray absorptiometry (DEXA). An individual is diagnosed with osteoporosis if their BMD (i.e. T score) is at least 2.5 standard deviations below the average BMD value for young healthy white women [37, 38]. Unfortunately, osteoporosis is often only diagnosed after an incident of a fracture or when the BMD is already very low. Moreover, 37.1% of male and 26.4% of female patients with a hip fracture die within one year [39]. Therefore, early detection and prevention are necessary. Pharmacological interventions consist mainly of bisphosphonates, strontium ranelate, raloxifene, denosumab, and parathyroid hormone peptides. These treatments are mostly effective, but adverse effects, including nausea, headache, skin conditions, and leg edema, are common [5].

Biomarkers of bone tissue turnover

Bone turnover markers have been identified and reviewed by others [40-42]. Most of the identified bone markers are cellular components of the bone matrix, with N-terminal propeptide of type 1 procollagen (PINP) and procollagen type 1 C-terminal propeptide (PICP) as markers of bone formation. Markers of bone resorption derived from cellular components of the bone matrix are C-terminal cross-linked telopeptide of type 1 collagen (CTX-I), N-terminal cross-linked telopeptide of type 1 collagen (NTX-I), type 1 collagen alpha 1 helicoidal peptide (HELP), deoxypyridinoline (DPD), and pyridinoline (PYD). Two other specific and sensitive markers of bone formation are bone-specific alkaline phosphatase (BSAP) and serum osteocalcin. Serum tartrate-resistant acid phosphatase – isoform 5b (TRAP5b) and cathepsin K are other markers of bone resorption. Receptor activator of nuclear factor κ-B ligand (RANKL) and osteoprotegerin (OPG) are two proteins produced by osteoblasts. Most of these biomarkers can be measured in serum, EDTA plasma or urine. Bone turnover markers are used in clinical trials for determining the efficacy and the response to osteoporosis treatments, but for most bone markers the predictive and diagnostic value is currently limited. Vasikaran *et al.* recommended to use serum CTX-I as reference biomarker of bone resorption and serum PINP as reference biomarker of bone formation [43]. These two of the most widely used biomarkers of bone turnover are by-products of forming or degrading the main component of bone, type 1 collagen. An overview of the selected proposed bone markers can be found in **Figure 2**.

CTX-I

In the process of bone resorption, bone collagen is broken down by cleavage of the cross-linked type 1 collagen by cathepsin K, which is expressed by osteoclasts. Subsequently, the N- and C-terminal cross-linked telopeptides of type 1 collagen (NTX-I and CTX-I) are released into the blood circulation, with CTX-I as the preferred marker of bone resorption. CTX-I could be measured in serum, EDTA plasma, or urine [44]. CTX-I is relatively stable at room temperature, especially in EDTA [45]. Serum CTX-I decreased with age in both men and women. However, CTX-I levels gradually increased in men after 40-50 years of age and increased in postmenopausal women. CTX-I levels were lower in premenopausal women than in men, but levels were highest in women after menopause [46-48]. CTX-I shows a circadian rhythm with its nadir in the afternoon around 14:00 hours and its maximum in the night around 5:00 hours [49, 50]. Food intake causes a reduction in CTX-I levels while fasting causes an increase in CTX-I levels, thereby strongly influencing the circadian rhythm of CTX-I [51-54]. In contrast, the circadian rhythm of CTX-I is not influenced by age, sex, postmenopausal status, bed rest of 5 days, by absence of a circadian rhythm of cortisol, by absence of a light-dark cycle

(blindness), or by low bone mass [53, 55]. Generally, biomarkers of bone resorption show a stronger circadian rhythm than biomarkers of bone formation [40, 42].

PINP

When bone is formed, osteoblasts secrete the precursor of type 1 collagen, i.e. type 1 procollagen. To form type 1 collagen, the carboxy and aminoterminal extension peptides are enzymatically cleaved off. These C- and N-terminal propeptides of type 1 procollagen (PICP and PINP) are waste products and released into the circulation. Both PICP and PINP are considered to reflect newly formed type 1 collagen. However, circulating PINP is most specific and is therefore considered as the key biomarker of bone formation. PINP can be measured in serum and EDTA plasma. PINP has a low intra individual variability, a good assay precision, and is stable at room temperature [43, 45, 56-58]. Studies on the 24-hour rhythm of PINP are conflicting. Two studies did not observe a 24-hour rhythm, while two other studies found somewhat higher levels in the night, albeit in men only in one of the two studies [55-58]. PINP levels were higher in men compared to premenopausal women, but levels were higher in postmenopausal women. With age, PINP levels decreased in both men and women, but increased after menopause in women [47, 48]. PINP levels were slightly influenced by food intake, for example when measured in the fed state or during an intravenous glucose tolerance test, PINP levels were somewhat lower, but oral glucose ingestion did not have a significant effect on PINP levels [59-61].

Osteocalcin

Besides collagen, osteoblasts also produce a protein called osteocalcin at sites of new bone formation. Osteocalcin is mostly incorporated into the bone matrix, but a small fraction is released into the circulation [62]. The specific function of osteocalcin remains debatable, but it is suggested to be involved in bone mineralization. Since the process of osteoid mineralization occurs in a late stadium of the bone turnover process, osteocalcin is a late marker of osteoblast activity. Furthermore, since osteocalcin is part of the bone matrix, osteocalcin fragments are also released into the circulation during bone resorption. Therefore osteocalcin is commonly used as a biomarker of bone turnover. Osteocalcin levels decreased with age in both men and women, but increased after menopause in women and increased slightly after age 65 for men. Osteocalcin levels were higher in young men than in premenopausal women, but lower in older men compared to women after menopause [46, 63, 64]. Serum osteocalcin shows a circadian rhythm with its nadir in the afternoon and its peak at night. Age, sex, or menopausal status did not influence this circadian rhythm [65-69].

Other (potential) biomarkers of bone tissue turnover
The most widely used bone markers, CTX-I, PINP, and osteocalcin, represent the function of osteoblasts or osteoclasts. However, the osteocyte is the most abundant cell type of bone tissue and plays a key regulatory role in bone and mineral homeostasis [70]. Therefore, markers of osteocyte activity are, although less established, promising novel biomarkers of bone turnover. Osteocytes are multifunctional cells which are differentiated osteoblasts, that control calcium and phosphate levels, and detect mechanical forces [70]. Osteocytes were found to play a key role in the regulation of bone turnover by producing factors including sclerostin and Dickkopf-related protein 1 (DKK1). These factors are negative regulators of bone formation that inhibit osteoblast activity via blocking the Wnt signaling pathway by antagonizing the Wnt/lipoprotein receptor-related protein 5 [71, 72]. Serum sclerostin levels were higher in men than in women and levels correlated positively with age [73-77]. No circadian rhythm of sclerostin has been observed, although sclerostin levels varied over 24 hours in one study [55, 56]. DKK1 levels were somewhat higher in older individuals than in younger individuals and levels were higher in female than in male geriatric patients [77, 78]. In one study, DKK1 levels were found to have a large interindividual and intraindividual variation, so more research is needed before DKK1 can be recommended as a reliable marker for diagnostic or research purposes [55]. Furthermore, although osteocytes were found to play a key role in the regulation of bone turnover, it is questionable whether sclerostin and DKK1 are specific markers of bone turnover. Since osteocytes are multifunctional cells which for example also control calcium and phosphate levels, sclerostin and DKK1 might be suitable as general markers of bone activity but these markers might not be specific enough for the processes of bone resorption and formation.

Cartilage
Cartilage tissue consists of chondrocytes surrounded by an extracellular matrix (ECM) which defines its properties. Three types of cartilage can be distinguished: elastic cartilage, hyaline cartilage and fibrocartilage. Articular cartilage is hyaline cartilage covering the joint surfaces. In hyaline cartilage, the ECM consists predominantly of collagen type 2, proteoglycan aggregates containing glycosaminoglycans (GAGs) and other non-collagenous proteins. Collagen stabilizes and strengthens the tissue [79], while the proteoglycans bind water through their negative charge, conferring flexibility and shock-absorbing properties [80], both vital to cartilage function. Turnover rate of articular cartilage ECM is generally slow but differs dramatically of each of its components; proteoglycans have an estimated half-life of 25 years [81], while the estimated half-life of collagen in cartilage equals 117 years [82].

Osteoarthritis

The integrity of articular cartilage can be disrupted, due to major trauma or age-related degenerative changes. If the disruption is larger than the repair capacity, osteoarthritis may develop. Osteoarthritis is characterized by damage and eventually loss of articular cartilage, as well as remodeling of subchondral bone and aberrant bone formation at the joints margins called osteophytes [83]. Patients often experience joint pain and stiffness, and in severe disease limitation of movement [6]. The definition used in literature for presence of osteoarthritis varies, from purely radiographical criteria for which the most commonly used is the Kellgren and Lawrence score [84] to various questionnaire scores on subjective complaints expressed by patients. In clinical practice, the diagnosis is commonly based on the combination of features in the medical history and physical examination, sometimes supplemented with diagnostic imaging [6]. The reported prevalence of osteoarthritis varies between studies, although the World Health Organization estimates a prevalence of 9.6% in men and 18.0% in women worldwide [85]. The established risk factors for osteoarthritis include obesity, high age and female sex [86], whereas the respective roles of smoking, bone density and dietary factors are still under debate [86]. With the increasing rates of obesity and the ageing of the population, a rise in incidence of osteoarthritis can be expected in the near future. The current treatment consists of analgesic prescription and lifestyle advise, and in late stage of disease surgical joint replacement [6], which is associated with high perioperative morbidity and persistent functional impairment after 12 months [87]. Because of the high burden of the disease symptoms and shortcomings of current treatment modalities, identification of people at risk to target for prevention is essential. Furthermore, new treatments could possibly slow down progression in pre-symptomatic patients to postpone or even prevent symptoms and surgical treatment. To identify patients who might benefit from these treatments and to evaluate effects of treatment, we need biomarkers that give an insight in the processes taking place in articular cartilage.

Biomarkers of cartilage turnover

Several biomarkers of articular cartilage turnover have been identified in the context of arthropathies, including osteoarthritis, rheumatoid arthritis and ankylosing spondylitis [41, 88]. These markers can be grouped based on the particular process they are associated with or whether they are collagen-derived or not. Most markers of cartilage turnover are based on collagen type 2 metabolism; markers for degradation include C-terminal cross-linked telopeptide of type 2 collagen (CTX-II) and type 2 collagen fragments (C2C), while the predominant markers for formation are the C- and N-terminal propeptide of type 2 procollagen (PIICP and PIINP) [41, 88, 89]. The main non-collagenous marker of cartilage turnover is cartilage oligomeric matrix protein (COMP), a glycoprotein constituent of articular cartilage [41, 90]. Additionally, some markers of aggrecan

degradation by matrix metalloproteinases (MMPs) and aggrecanases have been described such as keratin sulphate, which are mostly elevated after joint injury [41]. The main proposed biomarkers of cartilage turnover are listed in **Figure 2**.

COMP

The ECM of articular cartilage comprises numerous proteins, all contributing to optimum functionality of the joint. One of the regulatory constituents of cartilage ECM is cartilage oligomeric matrix protein (COMP), a large pentameric glycoprotein secreted by chondrocytes [91]. COMP interacts with several other ECM proteins, aiding matrix assembly and stability through binding collagen fibres [92]. Upon degradation of cartilage, COMP is released into the synovial fluid and consequently leaks into the circulation [93]. Although COMP was first discovered in cartilage, it is expressed in multiple other tissues including tendons, synovium, cardiomyocytes and activated platelets [91]. Nevertheless, COMP is one of the most robust biomarkers for osteoarthritis [94]. Measurement of COMP can occur in serum or plasma [95] and in synovial fluid [96], though collection of synovial fluid in healthy individuals proved difficult to perform [97]. Physical activity was found to increase circulating COMP levels acutely [97-99], and a second peak occurs five hours after exercise [99]. However, the effect of exercise is not as pronounced in individuals who exercise habitually [100, 101]. On the contrary, bed rest decreases circulating levels of COMP [99, 102], though serum levels normalize within one day after discontinuation of bed rest [102]. Because of its sensitivity to physical activity, most studies include a 24 to 36 hour limitation of exercise prior to the test day [98, 99] and a short resting period of 15 to 30 minutes immediately before drawing blood for COMP measurements to reduce effects of activities of daily living on circulating COMP levels [98, 99, 101].

CTX-II

Upon enzymatic degradation of collagen type 2, cross-linked C-telopeptide of collagen type 2 (CTX-II) is one of the collagen fragments released [103]. CTX-II is a very promising biomarker for cartilage degradation due to the high specificity of collagen type 2 for cartilage, and its strong correlation with joint diseases [104]. Measurement can be performed in plasma [105], urine [104], serum and synovial fluid [106]. Urinary excretion of CTX-II (uCTX-II) is the most common measurement, which is also very convenient considering the high stability of uCTX-II at room temperature and after multiple freeze-thaw cycles [104]. Generally, uCTX-II levels are lower in older than in young adults [107, 108]. Individuals with a higher BMI tend to have higher uCTX-II than lean individuals [107, 109, 110]. Levels of uCTX-II are higher in women than in men [107, 110]. Estrogen levels appear to influence uCTX-II, since the highest levels are observed in postmenopausal women [107, 111] and a decrease of uCTX-II was described with the use hormonal replacement therapy [107, 112] and selective estrogen receptor modulator [113]. Bisphosphonate use was shown to

decrease uCTX-II in postmenopausal women, with return to baseline levels after withdrawal of bisphosphonate treatment [114]. The presence of a circadian rhythm in uCTX-II is not established; one study did find a circadian rhythm similar to CTX-I with a nadir in the late afternoon and evening [110], while no rhythmicity was observed in an earlier study [104]. Seasonal variation in plasma levels of CTX-II was observed, with a peak in November and nadir in May, possibly explained through seasonal variation in serum vitamin D levels which influences bone metabolism and thereby indirectly collagen type II degradation [105].

PIINP

Type 2 collagen is first released as a procollagen during synthesis with propeptides at the N- and C-terminus, which are removed by specific proteinases before deposition into fibrils of the ECM [115]. The N-terminal propeptide of type 2 procollagen (PIINP) can be measured in synovial fluid and serum, and the concentrations reflect the anabolic state of the chondrocytes [116]. However, type 2 procollagen is synthesized in two variants due to alternative splicing, resulting in procollagen type 2A and type 2B [117]. The splicing is cell type specific: procollagen 2A appears predominantly in chondroprogenitor cells, whereas type 2B is mainly produced by mature chondrocytes [118]. Both splice variants have distinct N-terminal propeptides; PIIANP and PIIBNP respectively. In osteoarthritis patients, PIIANP was present in deep zone cartilage [119] and higher circulating levels of PIIANP were associated with slower progression of disease [120]. However, transcription of procollagen type 2A was only seen in osteoarthritic cartilage and not in healthy cartilage tissue [121]. Therefore, PIIANP may not be a suitable for turnover of healthy cartilage. PIIBNP would be a more appropriate biomarker of cartilage formation in healthy populations, regarding its origin from adult cartilage tissue. An ELISA assay specific for PIIBNP has been developed [122], and has recently been used to distinguish arthropathies [123, 124] and for early drug development [125, 126]. Even though further validation is still warranted, PIIBNP is a promising biomarker for collagen type 2 synthesis in healthy adult populations.

Skeletal muscle

Skeletal muscle protein synthesis and degradation are in constant balance. Each skeletal muscle cell contains parallel arrays of myofibrils, which are composed of hundreds or thousands repeating units called sarcomeres. A sarcomere is the structural unit of skeletal muscle and consists of smaller interdigitating myofilaments; thin and thick filaments. Thin filaments are polymers of proteins with actin as the main component and molecules of tropomyosin and troponin as regulatory proteins binding to actin. Myosin-II molecules are the primary component of thick filaments. The coordinated interaction among troponin, tropomyosin, and actin allows actin-myosin interactions to be regulated by changes in calcium concentration, with an increase in calcium concentration

triggering contraction of the muscle [34]. An imbalance between the rate of skeletal muscle protein synthesis and breakdown results in loss of skeletal muscle mass, which occurs during ageing, disease, or inactivity [127]. Bedrest particularly results in increased nitrogen excretion and to a lesser extent in whole-body protein breakdown, indicating that mainly protein synthesis is inhibited during bed rest [128, 129].

Sarcopenia

Sarcopenia is an age-related disease that is associated with loss of skeletal muscle mass in combination with loss of muscle strength and/or physical performance [7, 8]. Ageing is accompanied by changes in body composition comprising an increase in fat mass and a decrease in lean body mass, in particular a decrease in skeletal muscle and bone mass. The causes of sarcopenia are multifactorial, and risk factors for sarcopenia have been reviewed by others and were found to comprise, apart from chronological age, genetic heritability, chronic disease, inflammation, insulin resistance, low physical activity, insufficient protein intake, and low estrogen, testosterone, and vitamin D levels [7, 36]. Sarcopenia can be diagnosed when a patient's muscle mass is less than 20th percentile of values for healthy young adults, determined by 24-hour urinary creatinine (except in patients with renal insufficiency), or more indirect by bioelectrical impedance, DEXA or imaging techniques, in combination with muscle strength (low grip strength) and poor physical functioning determined by a gait speed of 0.8 m/s or less [8]. Sarcopenia biomarkers have been reviewed elsewhere [130]. Sarcopenia is a strong predictor of late-life disability [131, 132]. The prevalence of sarcopenia is estimated in community-dwelling adults aged 60 years and older at 10% of men and 10% of women worldwide, rising rapidly up to 50% of men and 43.8% of women at age 80 years and older [133]. Early detection and prevention are necessary. Muscle resistance training combined with amino acid-containing supplements is the preferred treatment to prevent (progression of) sarcopenia in older persons [134].

Biomarkers of muscle protein synthesis and breakdown

Several biological markers have been shown to be associated with skeletal muscle mass, strength, and function. However, most of these biomarkers are not muscle-specific, including hemoglobin, serum albumin, urinary creatinine, and inflammatory biomarkers such as circulating C-reactive protein (CRP), interleukin 6 (IL-6), and tumor necrosis factor-α (TNF-α) [135, 136]. Muscle-specific biomarkers are serum myostatin, which is a suppressor of muscle growth [137], and an isoform of serum creatine kinase (CK-MM), which is a muscle enzyme that is increased as a reaction to muscle damage, for example after exercise [138]. An overview of the main proposed biomarkers of muscle protein synthesis and breakdown is given in **Figure 2**.

3-methylhistidine
The rate of muscle protein breakdown can be determined by measuring the rate of excretion of 3-methylhistidine. 3-methylhistidine is an amino acid which is formed by post-translational methylation of histidine residues in the myofibrillar proteins actin and myosin and it is released after breakdown of both proteins [139, 140]. 3-methylhistidine is preferably measured in (24-hours) urine, but can also be detected in serum or plasma. Preferably, a meat-free diet is initiated around 3 days before measurements to reduce the effect of dietary 3-methylhistidine intake. However, one study showed that when using a tracer-based method this might not be necessary [141]. Levels of 3-metylhistidine were not responsive to exercise [142, 143].

PIIINP
A biomarker of skeletal muscle remodeling is plasma N-terminal propeptide of type 3 procollagen (PIIINP) [136, 144]. P3NP is a fragment released during final stages of collagen type 3 synthesis in soft connective tissues. During this process, N- and C-terminal portions of procollagen type 3 are removed by specific proteinases and these fragments are released in the circulation [145-147]. Consequently, PIIINP can be measured in serum with precision and accuracy. PIIINP levels are shown to respond to exercise, testosterone, and growth hormone (GH) [148-152].

Infusion of isotopically labelled amino acids
Another method to determine the rate of protein turnover in muscle is by infusion of isotopes [153, 154]. The general protocol for this method is to infuse isotopically labeled amino acids and subsequently measure their incorporation into muscle tissue. First, isotopically labeled amino acids such as phenylalanine and leucine are produced. Secondly, after taking a baseline blood sample and/or muscle biopsy, these amino acids are infused in the bloodstream via a catheter, in a single dose or, preferably, continuously. Subsequently, blood is withdrawn at specific time points or muscle biopsies are taken to determine the rate of muscle protein synthesis. Usually, this method is combined with a meat-free or protein controlled diet. This approach is a robust method, but a disadvantage of this method is that it is invasive, especially when muscle biopsies are taken.

Brain
Brain tissue comprises of a collection of different cell types including neurons, astrocytes, and microglia. Together with the extensive vasculature, they create a dynamic and highly interdependent entity. Although each component has its own function, neurons are considered the main functional component of the brain. Therefore, the neuronal cells have been studied most extensively. In rodents, adult neurogenesis was first demonstrated in the dentate gyrus [155], and later in the

subventricular zone [156] from which the new neurons also migrate to other areas of the brain [157]. However, in human brains the presence of adult neurogenesis is still under debate [158, 159]. If there is indeed limited or no production of new neurons in adults, then preservation of existent neurons is of crucial importance. Consequently, clearance of potentially neurotoxic substances and waste materials for optimal neuroprotection might be of greater importance to tissue homeostasis in the brain than cellular turnover.

Neurodegenerative disease

The hallmark of neurodegenerative diseases is progressive loss of neurons, accompanied by gradual development of cognitive impairments and motor disorders [9]. According to previous research in model organisms and in post-mortem studies of human brains the neuronal loss is often accompanied by accumulation of waste products, e.g. amyloid β [160], neurofibrillary tangles [161] and Lewy body inclusions [162]. What distinguishes the different disease entities is the localization of the most pronounced neuronal loss; e.g. for Alzheimer's disease the hippocampus and frontotemporal lobes are the most affected structures [163], while in Parkinson's disease the substantia nigra and the basal ganglia are predominantly affected [164]. The nature of the symptoms of different neurodegenerative diseases corresponds to the functional loss of anatomical region affected, however severity of anatomical disturbances do not correspond directly with severity of symptoms [165, 166]. Since the course of neurodegenerative diseases is generally gradual and slow, a long period of subclinical disease such as mild cognitive impairment can be observed but is also frequently missed due to the insidious onset [9]. Therefore, most degenerative diseases are diagnosed when severe damage has already occurred, because only by then clearly distinguishable functional decline has developed. The disease etiology has not been fully elucidated; apart from genetic predisposition, advanced age is still the major risk factor for neurodegenerative diseases [167]. Other risk factors include sedentary lifestyle, obesity, tobacco smoking and hypertension [168]. Currently no disease modifying therapies exist for neurodegenerative diseases, despite decades of pharmaceutical research [169]. This could be due to the advanced stage of disease and extensive brain damage at time of diagnosis, hence the repair capacities might be exhausted. Therefore, earlier detection of processes of neurodegeneration might identify a window of opportunity for disease modifying therapies.

Biomarkers of brain tissue maintenance

Research into early markers of neurodegenerative diseases has been conducted extensively, especially for Alzheimer's dementia [170]. However, human brain tissue is inaccessible for histological examination during life. Furthermore, measurements in serum or plasma do not always reflect the cerebral milieu

due to the blood brain barrier. Cerebrospinal fluid (CSF) is closer to the brain environment, though the acquisition via lumbar puncture is rather invasive. Therefore, most biomarkers for neuronal turnover are derived from translational research in model organisms, or are merely predictive markers mostly developed to predict incident dementia in individuals with or without mild cognitive impairment. Currently, the most promising markers for adult neurogenesis and neurodegeneration from translational research are circulating regulatory molecules such as microRNAs (miRNAs) [171], natriuretic peptides (NPs) [172], brain-derived neurotrophic factor (BDNF) [173], and glial cell line-derived neurotrophic factor (GDNF) [174, 175]. The predictive markers can be grouped to three categories; they are related to accumulation of misfolded proteins, progressive brain atrophy or energy metabolism. Accumulation of amyloid β_{42} ($A\beta_{42}$) and tau can be observed before brain atrophy occurs [176], and can be considered an early marker of disturbed tissue homeostasis. After prolonged net neurodegeneration, brain atrophy can be visualized using different imaging techniques [177]. For energy metabolism several metabolomic biomarkers have been associated with dementia risk, of which especially compounds from lipid metabolism appear to correlate with risk of dementia [178].

Brain atrophy

Radiological techniques can give unique insight in the anatomical and physiological features of the brain *in vivo*. Structural imaging, using computed tomography (CT) or preferably magnetic resonance imaging (MRI), can reveal several anatomical changes reflecting tissue damage and loss. Atrophy of the medial temporal lobe and hippocampus can be observed in Alzheimer-related pathology [179, 180], while in early stage Parkinson's disease atrophy of the substantia nigra and basal ganglia are the primary abnormalities [164]. More general anatomical abnormalities include signs of cerebral small vessel disease and loss of cortical thickness [177, 181]. Although the resolution of MRI scans has improved dramatically over the past decades from 0.5 Tesla to 7 Tesla [182], volumetric changes are only perceivable after a prolonged period of net tissue loss. Therefore, it is a relatively late marker of brain tissue damage. A more subtle instrument could be fluorodeoxyglucose (^{18}F-FDG) positron emission tomography (PET), which measures glucose consumption at the synaptic level as a reflection of neuronal activity [183]. However, quite some limitations currently remain for using ^{18}F-FDG-PET in research setting, including lack of quantitative analysis methods and distortion of the results when cortical atrophy is present [184, 185]. Generally, imaging techniques as biomarkers have several limitations. Most measures of atrophy correlate well with clinical outcomes on a group level, but on an individual level these correlations are weak [166]. Furthermore, CT and PET-scanning involve radiation exposure for the study participant, the required scanners are not yet

widely available and the economic costs for imaging technologies are relatively high.

$A\beta_{42}$ and tau
As neuroprotection might be more important than cellular turnover in maintenance of the brain, accumulation of potentially harmful waste products could also be used as a biomarker for tissue homeostasis. Accordingly, accumulation of misfolded proteins such as fibrillar Aβ and neurofibrillary tangles are among the earliest abnormalities in neurodegenerative processes [176]. It is hypothesized that these accumulated proteins are neurotoxic, especially oligomerization appears to contribute to neurotoxic effects of $A\beta_{42}$ and tau [160, 161].

Since histological examination for abnormalities is only possible post-mortem, alternative measurements were sought. Amyloid β can be measured in CSF and in plasma and by using targeted PET imaging [186, 187]. Counterintuitively, higher plaque burden of amyloid deposition on autopsy was associated with lower concentrations of $A\beta_{42}$ in the CSF [188, 189]. This could be explained as a sign of reduced clearance of amyloid β from the brain. Although $A\beta_{42}$ can also be measured in plasma, these levels do not reflect cortical burden of amyloid plaques [190]. Cerebral burden of amyloid β can also be measured using PET. The first method developed is Pittsburg Compound-B (PiB) PET [187]. PiB PET imaging is consistent with post-mortem amyloid β plaque burden [191, 192]. Since PiB is technically difficult to work with due to its short half-life of only 20 minutes, alternative ligands have been developed with similar imaging properties [193, 194]. For all measurements related to amyloid β, it is important to realize that there is a strong influence of age and ApoE ε4 genotype [195, 196].

For tau, the total tau concentration can be measured in CSF but also the phosphorylated segment. While tau is considered a rather general marker of axonal damage [197], phosphorylated tau (p-tau) is thought to be more specific for neurofibrillary tangles [198]. Nevertheless, higher CSF concentrations of both total tau (t-tau) and p-tau were associated with more neurofibrillary pathologic abnormalities on autopsy [189, 198]. As an alternative to CSF measurements, p-tau in plasma can also be used as a marker, however the results are not as consistent as in CSF [186]. For measurements of tau the factors of influence are not fully clarified, though ApoE ε4 genotype and possibly age and sex appear to associate with higher tau concentrations [199, 200].

Other (potential) biomarkers of brain tissue maintenance
Although measures of atrophy and cerebral accumulation of misfolded proteins have been investigated extensively, they are far from perfect biomarkers. Both markers represent a relatively late result of flawed processes, and the acquisition

of these markers is costly and currently lacking accuracy. Ideally, biomarkers of brain tissue homeostasis would closely represent ongoing processes in the brain. Regulatory molecules are the most promising candidates identified by translational research. For example miRNAs, which are short non-coding RNA molecules (up to 23 nucleotides) which regulate gene expression post-transcriptionally by repressing translation [201]. Several miRNAs appear to spatially and temporary regulate replication and differentiation of neurons [171, 202, 203]. However, validation of identified miRNA signals is scarce and many studies have shown discordant results [204]. Other potentially interesting regulatory molecules could be NPs, which were shown to play an essential role in fluid homeostasis and neuro-inflammation [172]. BDNF could also be of interest as a biomarker, though previous research results have been conflicting [173]. Considering the absence of any truly suitable marker reflecting processes of brain homeostasis, broader approaches for identifying biomarkers could be of help. Broad panels of metabolomics and proteomics are commonly utilized to identify new markers of neurodegenerative diseases [205]. This method has several advantages for use as a biomarker; the measurements can be performed in serum, plasma or urine, most platforms are quite robust and a combination of suitable markers can be used for a comprehensive approach [205-207]. However, the different platforms that are available vary greatly in number and type of metabolites and proteins measured, and the limited overlap between the platforms hampers comparison of different studies [205].

DISCUSSION

Here, we present the hypothesis that biomarkers of tissue maintenance represent a novel category of biomarkers of ageing, at the interface between biomarkers of the hallmarks of ageing and markers of age-related functional decline and disease. However, these biomarkers should be first measured in ageing studies to assess their validity before they can be used in clinic. A panel of biomarkers for bone, cartilage, muscle, and brain tissue maintenance is proposed and their advantages and limitations for use in (pre)clinical studies are discussed. Established biomarkers are available for bone tissue, but biomarkers for cartilage and muscle tissue are less established and should be investigated in further studies. For brain tissue maintenance, no suitable established biomarkers are currently available. **Figure 2** comprises an overview of the proposed biomarkers.

These biomarkers can be measured in future ageing research to determine whether these biomarkers are indeed potential early markers of the ageing process, and in particular age-related diseases including osteoporosis, osteoarthritis, sarcopenia, and neurodegenerative diseases. At this moment it is unknown whether it is beneficial to have elevated levels of biomarkers of

tissue maintenance or reduced levels (assuming that the amount of damage is equal). Higher levels of tissue turnover could indicate that the tissue is capable to regenerate, which is positive for health and lifespan. However, higher levels could also indicate that eventually the capacity of regeneration will decrease faster, leading to earlier stem cell exhaustion, and thus an earlier onset of age-related functional decline. Lower levels of tissue maintenance and repair could indicate that many cells are already in senescence, so fewer cells are able to regenerate, which is undesirable. On the other hand, lower levels could also indicate that the tissue is only regenerating itself when it is really necessary leading to prolonged ability for maintenance and repair, and thus increased lifespan. Moreover, it could be that it is beneficial to have elevated levels at young age but reduced levels at old age or the other way around. To determine which of these scenario's is most likely, the proposed biomarkers could for example be measured in humans with different rates of ageing. Subjects that could be included in these validation studies are healthy individuals of different chronological ages, people showing delayed biological ageing, and people showing accelerated biological ageing. In a group of healthy individuals with the same chronological age, biological age can be quantified by (sub)clinical biomarkers of ageing described by others, since most of these biomarkers are well-defined and highly correlated with ageing. Subsequently, the group could be divided into individuals with poor functional status, i.e. with high biological age, and individuals with good functional status, i.e. with low biological age. Their measured biomarkers of tissue regeneration can then be compared between the groups and linked to biological age.

Another design of a validation study is to include offspring of long-living families, since they are considered to have the genetic propensity to reach old age in good health like their parents, so they are more likely to be biologically younger and to show features of delayed ageing [208]. Studies in offspring of long-living families have shown that these individuals, compared to age-matched controls, are generally healthier and have a lower prevalence of age-related diseases [209]. Previously, we measured several biomarkers of bone tissue turnover in serially sampled blood obtained from a group of 37 middle-aged individuals, comprising offspring of long-living families and age-matched controls, and assessed the variation in serum concentration of these biomarkers over 24 hours [55]. In **Figure 3**, we present the data on markers of bone tissue turnover stratified for members from long-lived families and age-matched controls, as an example towards the use of biomarkers of tissue maintenance in a preclinical study. In this study, three out of the five bone markers seemed to be lower in offspring of long-lived families compared to controls. Given the small sample size of this preliminary study, we propose that larger studies will be required to address this hypothesis in greater depth. Furthermore, as suggested by Burkle *et al.*, people with characteristics of accelerated ageing, for example people with Down's syndrome or Werner's

syndrome, and possibly people with obesity, could also be included in biomarker-validation studies [12]. However, before validated biomarkers can be broadly used in research or the clinic, measurement of these biomarkers should be standardized [210].

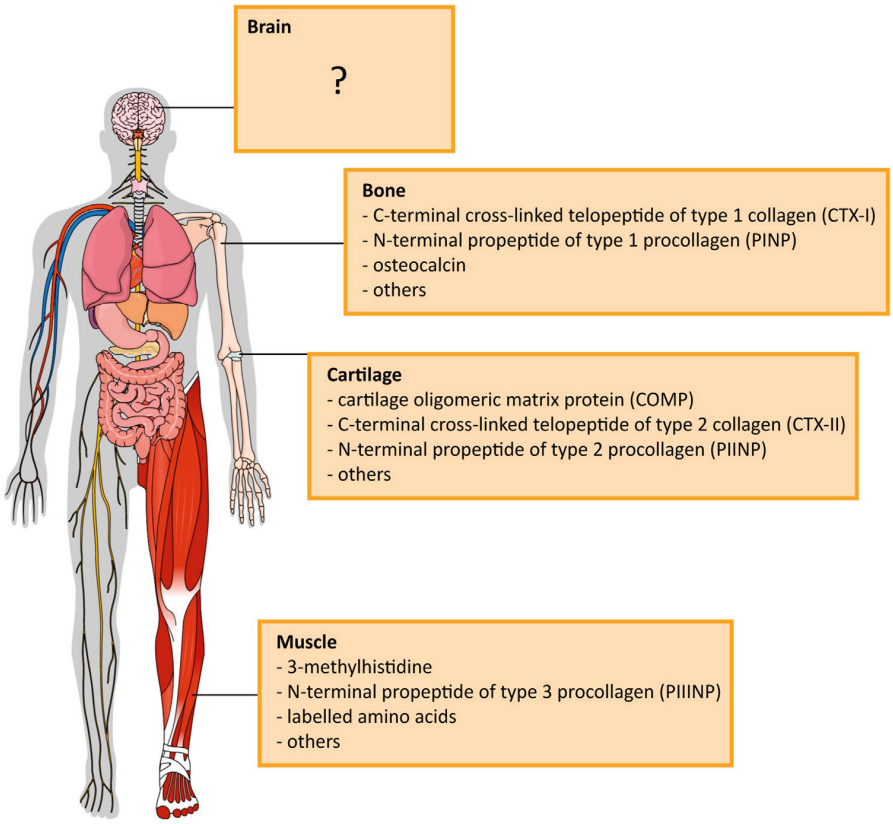

Figure 2. Overview of proposed biomarkers of tissue maintenance
Main proposed biomarkers of maintenance of bone, cartilage, and muscle tissue. This selection of biomarkers could be measured in future ageing research to determine whether these biomarkers are indeed potential early markers of the ageing process, and of age-related diseases including osteoporosis, osteoarthritis, and sarcopenia. For brain tissue maintenance no suitable biomarkers are currently available. Figure created in the Mind the Graph platform: www.mindthegraph.com.

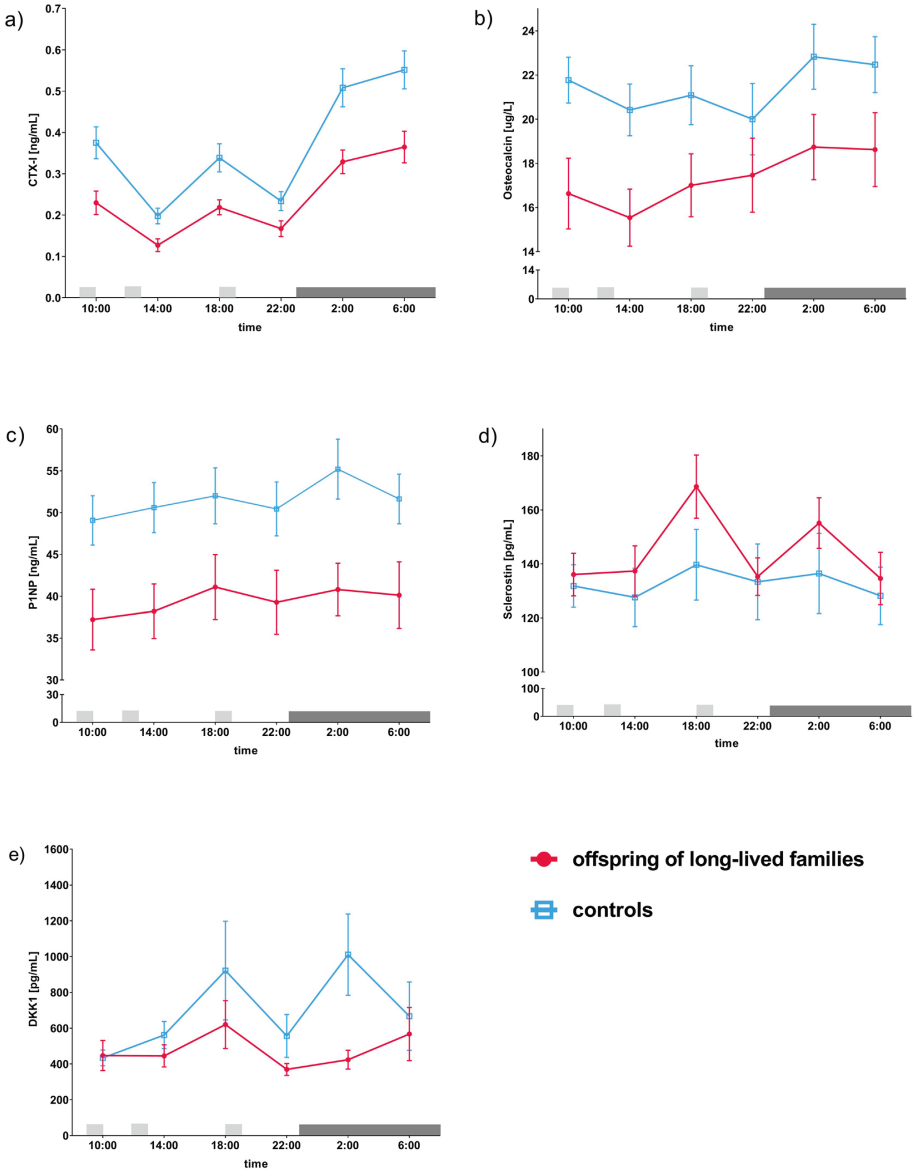

Figure 3. Bone markers over 24 h stratified for offspring of long-lived families and controls
The mean (SE) of a) C-terminal cross-linked telopeptide of type 1 collagen (CTX-I), b) osteocalcin, c) N-terminal propeptide of type 1 procollagen (PINP), d) sclerostin, and e) Dickkopf-related protein 1 (DKK1) are presented every 4 h starting at 10:00 during 24 h for 19 offspring of long-lived families (pink, dark) and 18 controls (blue, light). Light bars represent meal times and dark bars represent the period when the lights were switched off.

Whether reduced or elevated levels of biomarkers of tissue maintenance are beneficial might also be dependent on the context, including the (micro) environment, communication with other tissues and cells, and/or circulating factors. For example, other ageing processes, such as (immuno)senescence and inflammaging, were first seen as detrimental and reducing lifespan, but researchers recently proposed that these processes can also be viewed as adaptive responses leading to enhanced survival [30, 211]. Whether these processes are beneficial or detrimental depends on the microenvironment and circulating factors. Also parabiosis experiments demonstrate that circulating factors are important for proper functioning [212, 213]. In this review we presented some examples of internal and external factors that influence tissue maintenance, including hormones, inflammatory factors, nutrition, exercise, immobility, etc. For example, sex hormones are related to bone and muscle maintenance, but also growth factors as GH and IGF-1 strongly influence the regenerative capacity of tissues. Furthermore, the state of inflammaging influences many processes in the body, probably including tissue maintenance [214]. This shows that also tissue maintenance might be highly dependent on the (micro)environment and signaling/circulating factors. Therefore, when measuring biomarkers of tissue maintenance, one should consider to include other regulatory factors to get a more comprehensive picture. Moreover, these regulating factors might even be more informative than a single biomarker of tissue maintenance. For example, specific serum miRNA profiles were found to relate to bone pathologies with even a higher predictive power than bone mineral density or bone turnover markers [215]. Therefore, future research could besides validating proposed biomarkers of tissue maintenance, include identification of circulating factors crucial for tissue maintenance.

FUNDING

This work was supported by the European Commission project THYRAGE (Horizon 2020 research and innovation programme, 666869). E. van der Spoel was supported by a personal PhD grant from the Leiden University Medical Center.

REFERENCES

1. Partridge L, Deelen J, Slagboom PE. Facing up to the global challenges of ageing. *Nature*. Sep 2018;561(7721):45-56. doi:10.1038/s41586-018-0457-8
2. Mooijaart SP, Broekhuizen K, Trompet S, et al. Evidence-based medicine in older patients: how can we do better? *Neth J Med*. 6/2015 2015;73(5):211-218. Not in File.
3. Kirkwood TB, Austad SN. Why do we age? *Nature*. Nov 9 2000;408(6809):233-8. doi:10.1038/35041682
4. Fried TR, Tinetti ME, Iannone L, O'Leary JR, Towle V, Van Ness PH. Health outcome prioritization as a tool for decision making among older persons with multiple chronic conditions. *Arch Intern Med*. Nov 14 2011;171(20):1854-6. doi:10.1001/archinternmed.2011.424
5. Strom O, Borgstrom F, Kanis JA, et al. Osteoporosis: burden, health care provision and opportunities in the EU: a report prepared in collaboration with the International Osteoporosis Foundation (IOF) and the European Federation of Pharmaceutical Industry Associations (EFPIA). *Arch Osteoporos*. 2011;6:59-155. doi:10.1007/s11657-011-0060-1
6. Bijlsma JW, Berenbaum F, Lafeber FP. Osteoarthritis: an update with relevance for clinical practice. *Lancet (London, England)*. Jun 18 2011;377(9783):2115-26. doi:10.1016/s0140-6736(11)60243-2
7. Fielding RA, Vellas B, Evans WJ, et al. Sarcopenia: an undiagnosed condition in older adults. Current consensus definition: prevalence, etiology, and consequences. International working group on sarcopenia. *J Am Med Dir Assoc*. May 2011;12(4):249-56. doi:10.1016/j.jamda.2011.01.003
8. Cruz-Jentoft AJ, Baeyens JP, Bauer JM, et al. Sarcopenia: European consensus on definition and diagnosis: Report of the European Working Group on Sarcopenia in Older People. *Age Ageing*. Jul 2010;39(4):412-23. doi:10.1093/ageing/afq034
9. Ahmad K, Baig MH, Mushtaq G, Kamal MA, Greig NH, Choi I. Commonalities in Biological Pathways, Genetics, and Cellular Mechanism between Alzheimer Disease and Other Neurodegenerative Diseases: An In Silico-Updated Overview. *Current Alzheimer research*. 2017;14(11):1190-1197. doi:10.2174/1567205014666170203141151
10. Lara J, Cooper R, Nissan J, et al. A proposed panel of biomarkers of healthy ageing. *BMC Med*. 2015 2015;13:222. Not in File. doi:10.1186/s12916-015-0470-9 [doi];10.1186/s12916-015-0470-9 [pii]
11. Belsky DW, Caspi A, Houts R, et al. Quantification of biological aging in young adults. *Proc Natl Acad Sci U S A*. 7/28/2015 2015;112(30):E4104-E4110. Not in File. doi:1506264112 [pii];10.1073/pnas.1506264112 [doi]
12. Burkle A, Moreno-Villanueva M, Bernhard J, et al. MARK-AGE biomarkers of ageing. *Mech Ageing Dev*. 11/2015 2015;151:2-12. Not in File. doi:S0047-6374(15)00031-7 [pii];10.1016/j.mad.2015.03.006 [doi]
13. Sebastiani P, Thyagarajan B, Sun F, et al. Biomarker signatures of aging. *Aging Cell*. Apr 2017;16(2):329-338. doi:10.1111/acel.12557
14. Lopez-Otin C, Blasco MA, Partridge L, Serrano M, Kroemer G. The hallmarks of aging. *Cell*. 6/6/2013 2013;153(6):1194-1217. Not in File. doi:S0092-8674(13)00645-4 [pii];10.1016/j.cell.2013.05.039 [doi]
15. Lindahl A. Tissue homeostasis. Book Chapter. 2008:73-87.

16. WHO. World Health Organization International Programme on Chemical Safety Biomarkers in Risk Assessment: Validity and Validation. http://www.inchem.org/documents/ehc/ehc/ehc222.htm.
17. Cooper R, Kuh D, Hardy R, Mortality Review G, Falcon, Teams HAS. Objectively measured physical capability levels and mortality: systematic review and meta-analysis. *BMJ*. Sep 9 2010;341:c4467. doi:10.1136/bmj.c4467
18. Studenski S, Perera S, Patel K, et al. Gait speed and survival in older adults. *JAMA*. Jan 5 2011;305(1):50-8. doi:10.1001/jama.2010.1923
19. Takata Y, Ansai T, Soh I, et al. Cognitive function and 10 year mortality in an 85 year-old community-dwelling population. *Clin Interv Aging*. 2014;9:1691-9. doi:10.2147/CIA.S64107
20. Koopman JJ, van Bodegom D, van Heemst D, Westendorp RG. Handgrip strength, ageing and mortality in rural Africa. *Age Ageing*. May 2015;44(3):465-70. doi:10.1093/ageing/afu165
21. Khan SS, Singer BD, Vaughan DE. Molecular and physiological manifestations and measurement of aging in humans. *Aging Cell*. Aug 2017;16(4):624-633. doi:10.1111/acel.12601
22. Bai P. Biology of Poly(ADP-Ribose) Polymerases: The Factotums of Cell Maintenance. *Mol Cell*. Jun 18 2015;58(6):947-58. doi:10.1016/j.molcel.2015.01.034
23. Sanders JL, Newman AB. Telomere length in epidemiology: a biomarker of aging, age-related disease, both, or neither? *Epidemiol Rev*. 2013;35:112-31. doi:10.1093/epirev/mxs008
24. Jylhava J, Pedersen NL, Hagg S. Biological Age Predictors. *EBioMedicine*. Jul 2017;21:29-36. doi:10.1016/j.ebiom.2017.03.046
25. Horvath S, Raj K. DNA methylation-based biomarkers and the epigenetic clock theory of ageing. *Nat Rev Genet*. Jun 2018;19(6):371-384. doi:10.1038/s41576-018-0004-3
26. Longo VD, Finch CE. Evolutionary medicine: from dwarf model systems to healthy centenarians? *Science*. Feb 28 2003;299(5611):1342-6. doi:10.1126/science.1077991
27. Trout KK, Homko C, Tkacs NC. Methods of measuring insulin sensitivity. *Biol Res Nurs*. Apr 2007;8(4):305-18. doi:10.1177/1099800406298775
28. Hekimi S, Lapointe J, Wen Y. Taking a "good" look at free radicals in the aging process. *Trends Cell Biol*. Oct 2011;21(10):569-76. doi:10.1016/j.tcb.2011.06.008
29. Sharpless NE, Sherr CJ. Forging a signature of in vivo senescence. *Nat Rev Cancer*. Jul 2015;15(7):397-408. doi:10.1038/nrc3960
30. Munoz-Espin D, Serrano M. Cellular senescence: from physiology to pathology. *Nat Rev Mol Cell Biol*. Jul 2014;15(7):482-96. doi:10.1038/nrm3823
31. Mosaad YM. Hematopoietic stem cells: an overview. *Transfus Apher Sci*. Dec 2014;51(3):68-82. doi:10.1016/j.transci.2014.10.016
32. S. Melmed KSP, P.R. Larsen, H.M. Kronenberg, et al. Williams Textbook of Endocrinology, 13th Edition. Book. *Elsevier*. 2016:1335.
33. Kaufman JM, Vermeulen A. The decline of androgen levels in elderly men and its clinical and therapeutic implications. *Endocr Rev*. Oct 2005;26(6):833-76. doi:10.1210/er.2004-0013
34. Boron WFB, E.L. Medical Physiology: a Cellular and Molecular Approach, second edition. Book. *Saunders Elsevier*. 2009;
35. Ardawi MS, Rouzi AA, Qari MH. Physical activity in relation to serum sclerostin, insulin-like growth factor-1, and bone turnover markers in healthy premenopausal women: a cross-sectional and a longitudinal study. *J Clin Endocrinol Metab*. Oct 2012;97(10):3691-9. doi:10.1210/jc.2011-3361

36. Curtis E, Litwic A, Cooper C, Dennison E. Determinants of Muscle and Bone Aging. *J Cell Physiol*. 11/2015 2015;230(11):2618-2625. Not in File. doi:10.1002/jcp.25001 [doi]
37. NIH Consensus Development Panel. Osteoporosis Prevention, Diagnosis, and Therapy, March 7-29, 2000: highlights of the conference. *South Med J*. Jun 2001;94(6):569-73.
38. Rachner TD, Khosla S, Hofbauer LC. Osteoporosis: now and the future. *Lancet*. 4/9/2011 2011;377(9773):1276-1287. Not in File. doi:S0140-6736(10)62349-5 [pii];10.1016/S0140-6736(10)62349-5 [doi]
39. Kannegaard PN, van der Mark S, Eiken P, Abrahamsen B. Excess mortality in men compared with women following a hip fracture. National analysis of comedications, comorbidity and survival. *Age Ageing*. Mar 2010;39(2):203-9. doi:10.1093/ageing/afp221
40. Garnero P. The Utility of Biomarkers in Osteoporosis Management. *Mol Diagn Ther*. Aug 2017;21(4):401-418. doi:10.1007/s40291-017-0272-1
41. Woitge HW, Seibel MJ. Markers of Bone and Cartilage Turnover. *Experimental and clinical endocrinology & diabetes : official journal, German Society of Endocrinology [and] German Diabetes Association*. Jul 2017;125(7):454-469. doi:10.1055/s-0043-106438
42. Wheater G, Elshahaly M, Tuck SP, Datta HK, van Laar JM. The clinical utility of bone marker measurements in osteoporosis. *Journal of Translational Medicine*. 8/29/2013 2013;11Not in File.
43. Vasikaran S, Eastell R, Bruyere O, et al. Markers of bone turnover for the prediction of fracture risk and monitoring of osteoporosis treatment: a need for international reference standards. *Osteoporos Int*. 2/2011 2011;22(2):391-420. Not in File. doi:10.1007/s00198-010-1501-1 [doi]
44. Chubb SA. Measurement of C-terminal telopeptide of type I collagen (CTX) in serum. *Clin Biochem*. Aug 2012;45(12):928-35. doi:10.1016/j.clinbiochem.2012.03.035
45. Stokes FJ, Ivanov P, Bailey LM, Fraser WD. The effects of sampling procedures and storage conditions on short-term stability of blood-based biochemical markers of bone metabolism. *Clin Chem*. Jan 2011;57(1):138-40. doi:10.1373/clinchem.2010.157289
46. Ardawi MS, Maimani AA, Bahksh TA, Rouzi AA, Qari MH, Raddadi RM. Reference intervals of biochemical bone turnover markers for Saudi Arabian women: a cross-sectional study. *Bone*. Oct 2010;47(4):804-14. doi:10.1016/j.bone.2010.07.017
47. Jenkins N, Black M, Paul E, Pasco JA, Kotowicz MA, Schneider HG. Age-related reference intervals for bone turnover markers from an Australian reference population. *Bone*. Aug 2013;55(2):271-6. doi:10.1016/j.bone.2013.04.003
48. Michelsen J, Wallaschofski H, Friedrich N, et al. Reference intervals for serum concentrations of three bone turnover markers for men and women. *Bone*. Dec 2013;57(2):399-404. doi:10.1016/j.bone.2013.09.010
49. Dovio A, Generali D, Tampellini M, et al. Variations along the 24-hour cycle of circulating osteoprotegerin and soluble RANKL: a rhythmometric analysis. *Osteoporos Int*. Jan 2008;19(1):113-7. doi:10.1007/s00198-007-0423-z
50. Wichers M, Schmidt E, Bidlingmaier F, Klingmuller D. Diurnal rhythm of CrossLaps in human serum. *Clin Chem*. Oct 1999;45(10):1858-60.
51. Christgau S, Bitsch-Jensen O, Hanover Bjarnason N, et al. Serum CrossLaps for monitoring the response in individuals undergoing antiresorptive therapy. *Bone*. May 2000;26(5):505-11. doi:10.1016/S8756-3282(00)00248-9
52. Bjarnason NH, Henriksen EE, Alexandersen P, Christgau S, Henriksen DB, Christiansen C. Mechanism of circadian variation in bone resorption. *Bone*. Jan 2002;30(1):307-13.

53. Qvist P, Christgau S, Pedersen BJ, Schlemmer A, Christiansen C. Circadian variation in the serum concentration of C-terminal telopeptide of type I collagen (serum CTx): effects of gender, age, menopausal status, posture, daylight, serum cortisol, and fasting. *Bone*. Jul 2002;31(1):57-61.
54. Henriksen DB, Alexandersen P, Bjarnason NH, et al. Role of gastrointestinal hormones in postprandial reduction of bone resorption. *J Bone Miner Res*. Dec 2003;18(12):2180-9. doi:10.1359/jbmr.2003.18.12.2180
55. van der Spoel E, Oei N, Cachucho R, et al. The 24-hour serum profiles of bone markers in healthy older men and women. *Bone*. Oct 3 2018;doi:10.1016/j.bone.2018.10.002
56. Swanson C, Shea SA, Wolfe P, et al. 24-hour profile of serum sclerostin and its association with bone biomarkers in men. *Osteoporos Int*. Jul 26 2017;doi:10.1007/s00198-017-4162-5
57. Redmond J, Fulford AJ, Jarjou L, Zhou B, Prentice A, Schoenmakers I. Diurnal Rhythms of Bone Turnover Markers in Three Ethnic Groups. *J Clin Endocrinol Metab*. Aug 2016;101(8):3222-30. doi:10.1210/jc.2016-1183
58. Wolthers OD, Heuck C, Heickendorff L. Diurnal variations in serum and urine markers of type I and type III collagen turnover in children. *Clin Chem*. Sep 2001;47(9):1721-2.
59. Clowes JA, Hannon RA, Yap TS, Hoyle NR, Blumsohn A, Eastell R. Effect of feeding on bone turnover markers and its impact on biological variability of measurements. *Bone*. Jun 2002;30(6):886-90.
60. Yavropoulou MP, Tomos K, Tsekmekidou X, et al. Response of biochemical markers of bone turnover to oral glucose load in diseases that affect bone metabolism. *Eur J Endocrinol*. Jun 2011;164(6):1035-41. doi:10.1530/EJE-11-0128
61. Xiang SK, Wan JB, Jiang XH, Zhu YH, Ma JH, Hua F. Effect of Intravenous Glucose Tolerance Test on Bone Turnover Markers in Adults with Normal Glucose Tolerance. *Med Sci Monit*. Jul 22 2016;22:2602-7.
62. Lombardi G, Perego S, Luzi L, Banfi G. A four-season molecule: osteocalcin. Updates in its physiological roles. *Endocrine*. Mar 2015;48(2):394-404. doi:10.1007/s12020-014-0401-0
63. Midtby M, Magnus JH, Joakimsen RM. The Tromso Study: a population-based study on the variation in bone formation markers with age, gender, anthropometry and season in both men and women. *Osteoporos Int*. 2001;12(10):835-43. doi:10.1007/s001980170034
64. Hannemann A, Friedrich N, Spielhagen C, et al. Reference intervals for serum osteocalcin concentrations in adult men and women from the study of health in Pomerania. *BMC Endocr Disord*. Mar 13 2013;13:11. doi:10.1186/1472-6823-13-11
65. Pietschmann P, Resch H, Woloszczuk W, Willvonseder R. A circadian rhythm of serum osteocalcin levels in postmenopausal osteoporosis. *Eur J Clin Invest*. Jun 1990;20(3):310-2.
66. Pedersen BJ, Schlemmer A, Hassager C, Christiansen C. Changes in the carboxyl-terminal propeptide of type I procollagen and other markers of bone formation upon five days of bed rest. *Bone*. Jul 1995;17(1):91-5.
67. Greenspan SL, Dresner-Pollak R, Parker RA, London D, Ferguson L. Diurnal variation of bone mineral turnover in elderly men and women. *Calcif Tissue Int*. May 1997;60(5):419-23.
68. Eastell R, Reid DM, Compston J, et al. Secondary prevention of osteoporosis: when should a non-vertebral fracture be a trigger for action? *QJM*. Nov 2001;94(11):575-97.
69. Gundberg CM, Markowitz ME, Mizruchi M, Rosen JF. Osteocalcin in human serum: a circadian rhythm. *J Clin Endocrinol Metab*. Apr 1985;60(4):736-9. doi:10.1210/jcem-60-4-736

70. Dallas SL, Prideaux M, Bonewald LF. The osteocyte: an endocrine cell ... and more. *Endocr Rev*. Oct 2013;34(5):658-90. doi:10.1210/er.2012-1026
71. Bafico A, Liu G, Yaniv A, Gazit A, Aaronson SA. Novel mechanism of Wnt signalling inhibition mediated by Dickkopf-1 interaction with LRP6/Arrow. *Nat Cell Biol*. Jul 2001;3(7):683-6. doi:10.1038/35083081
72. Li X, Zhang Y, Kang H, et al. Sclerostin binds to LRP5/6 and antagonizes canonical Wnt signaling. *J Biol Chem*. May 20 2005;280(20):19883-7. doi:10.1074/jbc.M413274200
73. Ardawi MS, Al-Kadi HA, Rouzi AA, Qari MH. Determinants of serum sclerostin in healthy pre- and postmenopausal women. *J Bone Miner Res*. Dec 2011;26(12):2812-22. doi:10.1002/jbmr.479
74. Modder UI, Hoey KA, Amin S, et al. Relation of age, gender, and bone mass to circulating sclerostin levels in women and men. *J Bone Miner Res*. Feb 2011;26(2):373-9. doi:10.1002/jbmr.217
75. Amrein K, Amrein S, Drexler C, et al. Sclerostin and its association with physical activity, age, gender, body composition, and bone mineral content in healthy adults. *J Clin Endocrinol Metab*. Jan 2012;97(1):148-54. doi:10.1210/jc.2011-2152
76. Bhattoa HP, Wamwaki J, Kalina E, Foldesi R, Balogh A, Antal-Szalmas P. Serum sclerostin levels in healthy men over 50 years of age. *J Bone Miner Metab*. Sep 2013;31(5):579-84. doi:10.1007/s00774-013-0451-z
77. Dovjak P, Heinze G, Rainer A, Sipos W, Pietschmann P. Serum levels of Dickkopf-1 are a potential negative biomarker of survival in geriatric patients. *Exp Gerontol*. Oct 1 2017;96:104-109. doi:10.1016/j.exger.2017.06.002
78. Bradburn S, McPhee JS, Bagley L, et al. Association between osteocalcin and cognitive performance in healthy older adults. *Age Ageing*. Nov 2016;45(6):844-849. doi:10.1093/ageing/afw137
79. Gordon MK, Hahn RA. Collagens. *Cell and tissue research*. Jan 2010;339(1):247-57. doi:10.1007/s00441-009-0844-4
80. Chandran PL, Horkay F. Aggrecan, an unusual polyelectrolyte: review of solution behavior and physiological implications. *Acta biomaterialia*. Jan 2012;8(1):3-12. doi:10.1016/j.actbio.2011.08.011
81. Maroudas A, Bayliss MT, Uchitel-Kaushansky N, Schneiderman R, Gilav E. Aggrecan turnover in human articular cartilage: use of aspartic acid racemization as a marker of molecular age. *Archives of biochemistry and biophysics*. Feb 1 1998;350(1):61-71. doi:10.1006/abbi.1997.0492
82. Verzijl N, DeGroot J, Thorpe SR, et al. Effect of collagen turnover on the accumulation of advanced glycation end products. *The Journal of biological chemistry*. Dec 15 2000;275(50):39027-31. doi:10.1074/jbc.M006700200
83. Sharma AR, Jagga S, Lee SS, Nam JS. Interplay between cartilage and subchondral bone contributing to pathogenesis of osteoarthritis. *International journal of molecular sciences*. Sep 30 2013;14(10):19805-30. doi:10.3390/ijms141019805
84. Kellgren JH, Lawrence JS. Radiological assessment of osteo-arthrosis. *Annals of the rheumatic diseases*. Dec 1957;16(4):494-502.
85. Wittenauer R, Smith L, Aden K. Background paper 6.12 osteoarthritis. *World Health Organisation*. 2013;
86. Allen KD, Golightly YM. State of the evidence. *Current opinion in rheumatology*. May 2015;27(3):276-83. doi:10.1097/bor.0000000000000161
87. Walsh M, Woodhouse LJ, Thomas SG, Finch E. Physical impairments and functional limitations: a comparison of individuals 1 year after total knee arthroplasty with control subjects. *Physical therapy*. Mar 1998;78(3):248-58.

88. Charni-Ben Tabassi N, Garnero P. Monitoring cartilage turnover. *Current rheumatology reports*. Apr 2007;9(1):16-24.
89. Garvican ER, Vaughan-Thomas A, Innes JF, Clegg PD. Biomarkers of cartilage turnover. Part 1: Markers of collagen degradation and synthesis. *Veterinary journal (London, England : 1997)*. Jul 2010;185(1):36-42. doi:10.1016/j.tvjl.2010.04.011
90. Garvican ER, Vaughan-Thomas A, Clegg PD, Innes JF. Biomarkers of cartilage turnover. Part 2: Non-collagenous markers. *Veterinary journal (London, England : 1997)*. Jul 2010;185(1):43-9. doi:10.1016/j.tvjl.2010.04.012
91. Posey KL, Coustry F, Hecht JT. Cartilage oligomeric matrix protein: COMPopathies and beyond. *Matrix biology : journal of the International Society for Matrix Biology*. Mar 9 2018;doi:10.1016/j.matbio.2018.02.023
92. Acharya C, Yik JH, Kishore A, Van Dinh V, Di Cesare PE, Haudenschild DR. Cartilage oligomeric matrix protein and its binding partners in the cartilage extracellular matrix: interaction, regulation and role in chondrogenesis. *Matrix biology : journal of the International Society for Matrix Biology*. Jul 2014;37:102-11. doi:10.1016/j.matbio.2014.06.001
93. Neidhart M, Hauser N, Paulsson M, DiCesare PE, Michel BA, Hauselmann HJ. Small fragments of cartilage oligomeric matrix protein in synovial fluid and serum as markers for cartilage degradation. *British journal of rheumatology*. Nov 1997;36(11):1151-60.
94. Lotz M, Martel-Pelletier J, Christiansen C, et al. Republished: Value of biomarkers in osteoarthritis: current status and perspectives. *Postgraduate medical journal*. Mar 2014;90(1061):171-8. doi:10.1136/postgradmedj-2013-203726rep
95. Tufan AC, Satiroglu-Tufan NL, Jackson GC, Semerci CN, Solak S, Yagci B. Serum or plasma cartilage oligomeric matrix protein concentration as a diagnostic marker in pseudoachondroplasia: differential diagnosis of a family. *European journal of human genetics : EJHG*. Oct 2007;15(10):1023-8. doi:10.1038/sj.ejhg.5201882
96. Saxne T, Heinegard D. Cartilage oligomeric matrix protein: a novel marker of cartilage turnover detectable in synovial fluid and blood. *British journal of rheumatology*. Sep 1992;31(9):583-91.
97. Hyldahl RD, Evans A, Kwon S, et al. Running decreases knee intra-articular cytokine and cartilage oligomeric matrix concentrations: a pilot study. *European journal of applied physiology*. Dec 2016;116(11-12):2305-2314. doi:10.1007/s00421-016-3474-z
98. Niehoff A, Muller M, Bruggemann L, et al. Deformational behaviour of knee cartilage and changes in serum cartilage oligomeric matrix protein (COMP) after running and drop landing. *Osteoarthritis and cartilage*. Aug 2011;19(8):1003-10. doi:10.1016/j.joca.2011.04.012
99. Mundermann A, Dyrby CO, Andriacchi TP, King KB. Serum concentration of cartilage oligomeric matrix protein (COMP) is sensitive to physiological cyclic loading in healthy adults. *Osteoarthritis and cartilage*. Jan 2005;13(1):34-8. doi:10.1016/j.joca.2004.09.007
100. Vuolteenaho K, Leppanen T, Kekkonen R, Korpela R, Moilanen E. Running a marathon induces changes in adipokine levels and in markers of cartilage degradation--novel role for resistin. *PloS one*. 2014;9(10):e110481. doi:10.1371/journal.pone.0110481
101. Cattano NM, Driban JB, Barbe MF, Tierney RT, Amin M, Sitler MR. Biochemical Response to a Moderate Running Bout in Participants With or Without a History of Acute Knee Injury. *Journal of athletic training*. Jun 2 2017;52(6):567-574. doi:10.4085/1062-6050-51.5.09

102. Liphardt AM, Mundermann A, Andriacchi TP, Achtzehn S, Heer M, Mester J. Sensitivity of serum concentration of cartilage biomarkers to 21-days of bed rest. *Journal of orthopaedic research : official publication of the Orthopaedic Research Society*. May 2018;36(5):1465-1471. doi:10.1002/jor.23786
103. Charni-Ben Tabassi N, Desmarais S, Bay-Jensen AC, Delaisse JM, Percival MD, Garnero P. The type II collagen fragments Helix-II and CTX-II reveal different enzymatic pathways of human cartilage collagen degradation. *Osteoarthritis and cartilage*. Oct 2008;16(10):1183-91. doi:10.1016/j.joca.2008.02.008
104. Christgau S, Garnero P, Fledelius C, et al. Collagen type II C-telopeptide fragments as an index of cartilage degradation. *Bone*. Sep 2001;29(3):209-15.
105. Brayda-Bruno M, Vigano M, Cauci S, et al. Plasma vitamin D and osteo-cartilaginous markers in Italian males affected by intervertebral disc degeneration: Focus on seasonal and pathological trend of type II collagen degradation. *Clinica chimica acta; international journal of clinical chemistry*. Aug 2017;471:87-93. doi:10.1016/j.cca.2017.05.028
106. Lohmander LS, Atley LM, Pietka TA, Eyre DR. The release of crosslinked peptides from type II collagen into human synovial fluid is increased soon after joint injury and in osteoarthritis. *Arthritis and rheumatism*. Nov 2003;48(11):3130-9. doi:10.1002/art.11326
107. Mouritzen U, Christgau S, Lehmann HJ, Tanko LB, Christiansen C. Cartilage turnover assessed with a newly developed assay measuring collagen type II degradation products: influence of age, sex, menopause, hormone replacement therapy, and body mass index. *Annals of the rheumatic diseases*. Apr 2003;62(4):332-6.
108. Rotterud JH, Reinholt FP, Beckstrom KJ, Risberg MA, Aroen A. Relationship between CTX-II and patient characteristics, patient-reported outcome, muscle strength, and rehabilitation in patients with a focal cartilage lesion of the knee: a prospective exploratory cohort study of 48 patients. *BMC musculoskeletal disorders*. Mar 24 2014;15:99. doi:10.1186/1471-2474-15-99
109. Holinka CF, Christiansen C, Tian XW, Ausmanas MK. Ethnic differences in levels of bone and cartilage biomarkers and hormonal responsiveness in nine groups of postmenopausal Asian women: the Pan-Asia Menopause (PAM) study. *Climacteric : the journal of the International Menopause Society*. Feb 2008;11(1):44-54. doi:10.1080/13697130701744886
110. Karsdal MA, Byrjalsen I, Bay-Jensen AC, Henriksen K, Riis BJ, Christiansen C. Biochemical markers identify influences on bone and cartilage degradation in osteoarthritis--the effect of sex, Kellgren-Lawrence (KL) score, body mass index (BMI), oral salmon calcitonin (sCT) treatment and diurnal variation. *BMC musculoskeletal disorders*. Jun 17 2010;11:125. doi:10.1186/1471-2474-11-125
111. Bay-Jensen AC, Tabassi NC, Sondergaard LV, et al. The response to oestrogen deprivation of the cartilage collagen degradation marker, CTX-II, is unique compared with other markers of collagen turnover. *Arthritis research & therapy*. 2009;11(1):R9.
112. Ausmanas MK, Holinka CF, Ling YS, Christiansen C. Effect of three doses of conjugated estrogens/medroxyprogesterone acetate on biomarkers of bone turnover and cartilage degradation in postmenopausal women: The Pan-Asia Menopause (PAM) study. *Climacteric : the journal of the International Menopause Society*. Aug 2007;10(4):306-13. doi:10.1080/13697130701463594
113. Christgau S, Tanko LB, Cloos PA, et al. Suppression of elevated cartilage turnover in postmenopausal women and in ovariectomized rats by estrogen and a selective estrogen-receptor modulator (SERM). *Menopause (New York, NY)*. Sep-Oct 2004;11(5):508-18.

114. Corsetti R, Perego S, Sansoni V, et al. Osteocartilaginous metabolic markers change over a 3-week stage race in pro-cyclists. *Scandinavian journal of clinical and laboratory investigation*. Oct 2015;75(6):523-30. doi:10.3109/00365513.2015.1057762
115. Peltonen L, Halila R, Ryhanen L. Enzymes converting procollagens to collagens. *Journal of cellular biochemistry*. 1985;28(1):15-21. doi:10.1002/jcb.240280104
116. Olsen AK, Sondergaard BC, Byrjalsen I, et al. Anabolic and catabolic function of chondrocyte ex vivo is reflected by the metabolic processing of type II collagen. *Osteoarthritis and cartilage*. Mar 2007;15(3):335-42. doi:10.1016/j.joca.2006.08.015
117. Ryan MC, Sandell LJ. Differential expression of a cysteine-rich domain in the amino-terminal propeptide of type II (cartilage) procollagen by alternative splicing of mRNA. *The Journal of biological chemistry*. Jun 25 1990;265(18):10334-9.
118. Sandell LJ, Morris N, Robbins JR, Goldring MB. Alternatively spliced type II procollagen mRNAs define distinct populations of cells during vertebral development: differential expression of the amino-propeptide. *The Journal of cell biology*. Sep 1991;114(6):1307-19.
119. Bay-Jensen AC, Andersen TL, Charni-Ben Tabassi N, et al. Biochemical markers of type II collagen breakdown and synthesis are positioned at specific sites in human osteoarthritic knee cartilage. *Osteoarthritis and cartilage*. May 2008;16(5):615-23. doi:10.1016/j.joca.2007.09.006
120. Berry PA, Maciewicz RA, Wluka AE, et al. Relationship of serum markers of cartilage metabolism to imaging and clinical outcome measures of knee joint structure. *Annals of the rheumatic diseases*. Oct 2010;69(10):1816-22. doi:10.1136/ard.2009.124420
121. Aigner T, Zhu Y, Chansky HH, Matsen FA, 3rd, Maloney WJ, Sandell LJ. Reexpression of type IIA procollagen by adult articular chondrocytes in osteoarthritic cartilage. *Arthritis and rheumatism*. Jul 1999;42(7):1443-50. doi:10.1002/1529-0131(199907)42:7<1443::aid-anr18>3.0.co;2-a
122. Gudmann NS, Wang J, Hoielt S, et al. Cartilage turnover reflected by metabolic processing of type II collagen: a novel marker of anabolic function in chondrocytes. *International journal of molecular sciences*. Oct 17 2014;15(10):18789-803. doi:10.3390/ijms151018789
123. Hua B, Olsen EHN, Sun S, et al. Serological biomarkers detect active joint destruction and inflammation in patients with haemophilic arthropathy. *Haemophilia : the official journal of the World Federation of Hemophilia*. Jul 2017;23(4):e294-e300. doi:10.1111/hae.13196
124. Gudmann NS, Munk HL, Christensen AF, et al. Chondrocyte activity is increased in psoriatic arthritis and axial spondyloarthritis. *Arthritis research & therapy*. Jun 16 2016;18(1):141. doi:10.1186/s13075-016-1040-z
125. Reker D, Kjelgaard-Petersen CF, Siebuhr AS, et al. Sprifermin (rhFGF18) modulates extracellular matrix turnover in cartilage explants ex vivo. *Journal of translational medicine*. Dec 12 2017;15(1):250. doi:10.1186/s12967-017-1356-8
126. Kjelgaard-Petersen CF, Sharma N, Kayed A, et al. Tofacitinib and TPCA-1 exert chondroprotective effects on extracellular matrix turnover in bovine articular cartilage ex vivo. *Biochemical pharmacology*. Jul 29 2018;doi:10.1016/j.bcp.2018.07.034
127. Evans WJ. Skeletal muscle loss: cachexia, sarcopenia, and inactivity. *Am J Clin Nutr*. Apr 2010;91(4):1123S-1127S. doi:10.3945/ajcn.2010.28608A
128. Ferrando AA, Lane HW, Stuart CA, Davis-Street J, Wolfe RR. Prolonged bed rest decreases skeletal muscle and whole body protein synthesis. *Am J Physiol*. Apr 1996;270(4 Pt 1):E627-33. doi:10.1152/ajpendo.1996.270.4.E627

129. Paddon-Jones D, Sheffield-Moore M, Cree MG, et al. Atrophy and impaired muscle protein synthesis during prolonged inactivity and stress. *J Clin Endocrinol Metab*. Dec 2006;91(12):4836-41. doi:10.1210/jc.2006-0651
130. Kalinkovich A, Livshits G. Sarcopenia--The search for emerging biomarkers. *Ageing Res Rev*. Jul 2015;22:58-71. doi:10.1016/j.arr.2015.05.001
131. Doherty TJ. Invited review: Aging and sarcopenia. *J Appl Physiol (1985)*. Oct 2003;95(4):1717-27. doi:10.1152/japplphysiol.00347.2003
132. Janssen I, Heymsfield SB, Ross R. Low relative skeletal muscle mass (sarcopenia) in older persons is associated with functional impairment and physical disability. *J Am Geriatr Soc*. May 2002;50(5):889-96.
133. Shafiee G, Keshtkar A, Soltani A, Ahadi Z, Larijani B, Heshmat R. Prevalence of sarcopenia in the world: a systematic review and meta- analysis of general population studies. *J Diabetes Metab Disord*. 2017;16:21. doi:10.1186/s40200-017-0302-x
134. Fiatarone MA, O'Neill EF, Ryan ND, et al. Exercise training and nutritional supplementation for physical frailty in very elderly people. *N Engl J Med*. Jun 23 1994;330(25):1769-75. doi:10.1056/NEJM199406233302501
135. Schaap LA, Pluijm SM, Deeg DJ, Visser M. Inflammatory markers and loss of muscle mass (sarcopenia) and strength. *Am J Med*. Jun 2006;119(6):526 e9-17. doi:10.1016/j.amjmed.2005.10.049
136. Cesari M, Fielding RA, Pahor M, et al. Biomarkers of sarcopenia in clinical trials-recommendations from the International Working Group on Sarcopenia. *J Cachexia Sarcopenia Muscle*. Sep 2012;3(3):181-90. doi:10.1007/s13539-012-0078-2
137. Lee SJ. Regulation of muscle mass by myostatin. *Annu Rev Cell Dev Biol*. 2004;20:61-86. doi:10.1146/annurev.cellbio.20.012103.135836
138. Brancaccio P, Maffulli N, Limongelli FM. Creatine kinase monitoring in sport medicine. *Br Med Bull*. 2007;81-82:209-30. doi:10.1093/bmb/ldm014
139. Long CL, Haverberg LN, Young VR, Kinney JM, Munro HN, Geiger JW. Metabolism of 3-methylhistidine in man. *Metabolism*. Aug 1975;24(8):929-35.
140. Sugden PH, Fuller SJ. Regulation of protein turnover in skeletal and cardiac muscle. *Biochem J*. Jan 01 1991;273(Pt 1):21-37.
141. Sheffield-Moore M, Dillon EL, Randolph KM, et al. Isotopic decay of urinary or plasma 3-methylhistidine as a potential biomarker of pathologic skeletal muscle loss. *J Cachexia Sarcopenia Muscle*. Mar 2014;5(1):19-25. doi:10.1007/s13539-013-0117-7
142. Hansen M, Trappe T, Crameri RM, Qvortrup K, Kjaer M, Langberg H. Myofibrillar proteolysis in response to voluntary or electrically stimulated muscle contractions in humans. *Scand J Med Sci Sports*. Feb 2009;19(1):75-82. doi:10.1111/j.1600-0838.2007.00766.x
143. Calles-Escandon J, Cunningham JJ, Snyder P, et al. Influence of exercise on urea, creatinine, and 3-methylhistidine excretion in normal human subjects. *Am J Physiol*. Apr 1984;246(4 Pt 1):E334-8. doi:10.1152/ajpendo.1984.246.4.E334
144. Curcio F, Ferro G, Basile C, et al. Biomarkers in sarcopenia: A multifactorial approach. *Exp Gerontol*. Dec 1 2016;85:1-8. doi:10.1016/j.exger.2016.09.007
145. Prockop DJ, Kivirikko KI, Tuderman L, Guzman NA. The biosynthesis of collagen and its disorders (first of two parts). *N Engl J Med*. Jul 5 1979;301(1):13-23. doi:10.1056/NEJM197907053010104
146. Niemela O. Radioimmunoassays for type III procollagen amino-terminal peptides in humans. *Clin Chem*. Aug 1985;31(8):1301-4.
147. Niemela O, Risteli L, Parkkinen J, Risteli J. Purification and characterization of the N-terminal propeptide of human type III procollagen. *Biochem J*. Nov 15 1985;232(1):145-50.

148. Bhasin S, He EJ, Kawakubo M, et al. N-terminal propeptide of type III procollagen as a biomarker of anabolic response to recombinant human GH and testosterone. *J Clin Endocrinol Metab*. Nov 2009;94(11):4224-33. doi:10.1210/jc.2009-1434
149. Wallace JD, Cuneo RC, Lundberg PA, et al. Responses of markers of bone and collagen turnover to exercise, growth hormone (GH) administration, and GH withdrawal in trained adult males. *J Clin Endocrinol Metab*. Jan 2000;85(1):124-33. doi:10.1210/jcem.85.1.6262
150. Crameri RM, Langberg H, Teisner B, et al. Enhanced procollagen processing in skeletal muscle after a single bout of eccentric loading in humans. *Matrix Biol*. Jul 2004;23(4):259-64. doi:10.1016/j.matbio.2004.05.009
151. Powrie JK, Bassett EE, Rosen T, et al. Detection of growth hormone abuse in sport. *Growth Horm IGF Res*. Jun 2007;17(3):220-6. doi:10.1016/j.ghir.2007.01.011
152. Chen F, Lam R, Shaywitz D, et al. Evaluation of early biomarkers of muscle anabolic response to testosterone. *J Cachexia Sarcopenia Muscle*. Mar 2011;2(1):45-56. doi:10.1007/s13539-011-0021-y
153. Rennie MJ, Smith K, Watt PW. Measurement of human tissue protein synthesis: an optimal approach. *Am J Physiol*. Mar 1994;266(3 Pt 1):E298-307.
154. Atherton PJ, Phillips BE, Wilkinson DJ. Exercise and Regulation of Protein Metabolism. *Prog Mol Biol Transl Sci*. 2015;135:75-98. doi:10.1016/bs.pmbts.2015.06.015
155. Altman J, Das GD. Autoradiographic and histological evidence of postnatal hippocampal neurogenesis in rats. *The Journal of comparative neurology*. Jun 1965;124(3):319-35.
156. Lois C, Alvarez-Buylla A. Proliferating subventricular zone cells in the adult mammalian forebrain can differentiate into neurons and glia. *Proceedings of the National Academy of Sciences of the United States of America*. Mar 1 1993;90(5):2074-7.
157. Lennington JB, Yang Z, Conover JC. Neural stem cells and the regulation of adult neurogenesis. *Reproductive biology and endocrinology : RB&E*. Nov 13 2003;1:99. doi:10.1186/1477-7827-1-99
158. Sorrells SF, Paredes MF, Cebrian-Silla A, et al. Human hippocampal neurogenesis drops sharply in children to undetectable levels in adults. *Nature*. 03/07/online 2018;555:377. doi:10.1038/nature25975
https://www.nature.com/articles/nature25975#supplementary-information
159. Bergmann O, Spalding KL, Frisen J. Adult Neurogenesis in Humans. *Cold Spring Harbor perspectives in biology*. Jul 1 2015;7(7):a018994. doi:10.1101/cshperspect.a018994
160. Hardy J, Selkoe DJ. The amyloid hypothesis of Alzheimer's disease: progress and problems on the road to therapeutics. *Science (New York, NY)*. Jul 19 2002;297(5580):353-6. doi:10.1126/science.1072994
161. Ballatore C, Lee VM, Trojanowski JQ. Tau-mediated neurodegeneration in Alzheimer's disease and related disorders. *Nature reviews Neuroscience*. Sep 2007;8(9):663-72. doi:10.1038/nrn2194
162. Arnaoutoglou NA, O'Brien JT, Underwood BR. Dementia with Lewy bodies - from scientific knowledge to clinical insights. *Nature reviews Neurology*. Dec 17 2018;doi:10.1038/s41582-018-0107-7
163. Pini L, Pievani M, Bocchetta M, et al. Brain atrophy in Alzheimer's Disease and aging. *Ageing research reviews*. Sep 2016;30:25-48. doi:10.1016/j.arr.2016.01.002
164. Zeighami Y, Fereshtehnejad SM, Dadar M, et al. A clinical-anatomical signature of Parkinson's disease identified with partial least squares and magnetic resonance imaging. *NeuroImage*. Dec 19 2017;doi:10.1016/j.neuroimage.2017.12.050

165. Pietzuch M, King AE, Ward DD, Vickers JC. The Influence of Genetic Factors and Cognitive Reserve on Structural and Functional Resting-State Brain Networks in Aging and Alzheimer's Disease. *Frontiers in aging neuroscience*. 2019;11:30. doi:10.3389/fnagi.2019.00030
166. Cholerton B, Larson EB, Quinn JF, et al. Precision Medicine: Clarity for the Complexity of Dementia. *The American Journal of Pathology*. 2016/03/01/ 2016;186(3):500-506. doi:https://doi.org/10.1016/j.ajpath.2015.12.001
167. WHO. *Global action plan on the public health response to dementia 2017-2025*. *https://apps.who.int/iris/bitstream/handle/10665/259615/9789241513487-eng.pdf?sequence=1*. 2017.
168. Livingston G, Sommerlad A, Orgeta V, et al. Dementia prevention, intervention, and care. *The Lancet*. 2017/12/16/ 2017;390(10113):2673-2734. doi:https://doi.org/10.1016/S0140-6736(17)31363-6
169. Cummings JL, Tong G, Ballard C. Treatment Combinations for Alzheimer's Disease: Current and Future Pharmacotherapy Options. *Journal of Alzheimer's disease : JAD*. 2019;67(3):779-794. doi:10.3233/JAD-180766
170. Molinuevo JL, Ayton S, Batrla R, et al. Current state of Alzheimer's fluid biomarkers. *Acta neuropathologica*. Dec 2018;136(6):821-853. doi:10.1007/s00401-018-1932-x
171. Stappert L, Klaus F, Brustle O. MicroRNAs Engage in Complex Circuits Regulating Adult Neurogenesis. *Frontiers in neuroscience*. 2018;12:707. doi:10.3389/fnins.2018.00707
172. Mahinrad S, de Craen AJM, Yasar S, van Heemst D, Sabayan B. Natriuretic peptides in the central nervous system: Novel targets for cognitive impairment. *Neuroscience and biobehavioral reviews*. Sep 2016;68:148-156. doi:10.1016/j.neubiorev.2016.05.022
173. Numakawa T, Odaka H, Adachi N. Actions of Brain-Derived Neurotrophin Factor in the Neurogenesis and Neuronal Function, and Its Involvement in the Pathophysiology of Brain Diseases. *International journal of molecular sciences*. Nov 19 2018;19(11) doi:10.3390/ijms19113650
174. Airaksinen MS, Saarma M. The GDNF family: signalling, biological functions and therapeutic value. *Nature reviews Neuroscience*. May 2002;3(5):383-94. doi:10.1038/nrn812
175. Kim M, Kim DJ. GFRA1: A Novel Molecular Target for the Prevention of Osteosarcoma Chemoresistance. *International journal of molecular sciences*. Apr 4 2018;19(4) doi:10.3390/ijms19041078
176. Jack CR, Jr., Knopman DS, Jagust WJ, et al. Tracking pathophysiological processes in Alzheimer's disease: an updated hypothetical model of dynamic biomarkers. *The Lancet Neurology*. Feb 2013;12(2):207-16. doi:10.1016/s1474-4422(12)70291-0
177. Dickerson BC, Wolk DA. MRI cortical thickness biomarker predicts AD-like CSF and cognitive decline in normal adults. *Neurology*. Jan 10 2012;78(2):84-90. doi:10.1212/WNL.0b013e31823efc6c
178. Mapstone M, Cheema AK, Fiandaca MS, et al. Plasma phospholipids identify antecedent memory impairment in older adults. *Nature medicine*. Apr 2014;20(4):415-8. doi:10.1038/nm.3466
179. Grundman M, Sencakova D, Jack CR, Jr., et al. Brain MRI hippocampal volume and prediction of clinical status in a mild cognitive impairment trial. *Journal of molecular neuroscience : MN*. Aug-Oct 2002;19(1-2):23-7. doi:10.1007/s12031-002-0006-6
180. DeCarli C, Frisoni GB, Clark CM, et al. Qualitative estimates of medial temporal atrophy as a predictor of progression from mild cognitive impairment to dementia. *Archives of neurology*. Jan 2007;64(1):108-15. doi:10.1001/archneur.64.1.108
181. Zwanenburg JJM, van Osch MJP. Targeting Cerebral Small Vessel Disease With MRI. *Stroke*. Nov 2017;48(11):3175-3182. doi:10.1161/strokeaha.117.016996

182. Zwanenburg JJ, van der Kolk AG, Luijten PR. Ultra-High-Field MR Imaging: Research Tool or Clinical Need? *PET clinics*. Jul 2013;8(3):311-28. doi:10.1016/j.cpet.2013.03.004
183. Gallivanone F, Della Rosa PA, Castiglioni I. Statistical Voxel-Based Methods and [18F] FDG PET Brain Imaging: Frontiers for the Diagnosis of AD. *Current Alzheimer research*. 2016;13(6):682-94.
184. Bonte S, Vandemaele P, Verleden S, et al. Healthy brain ageing assessed with 18F-FDG PET and age-dependent recovery factors after partial volume effect correction. *European journal of nuclear medicine and molecular imaging*. May 2017;44(5):838-849. doi:10.1007/s00259-016-3569-0
185. Sarikaya I, Sarikaya A, Elgazzar AH. Current Status of (18)F-FDG PET Brain Imaging in Patients with Dementia. *Journal of nuclear medicine technology*. Dec 2018;46(4):362-367. doi:10.2967/jnmt.118.210237
186. Olsson B, Lautner R, Andreasson U, et al. CSF and blood biomarkers for the diagnosis of Alzheimer's disease: a systematic review and meta-analysis. *The Lancet Neurology*. Jun 2016;15(7):673-684. doi:10.1016/s1474-4422(16)00070-3
187. Klunk WE, Engler H, Nordberg A, et al. Imaging brain amyloid in Alzheimer's disease with Pittsburgh Compound-B. *Annals of neurology*. Mar 2004;55(3):306-19. doi:10.1002/ana.20009
188. Strozyk D, Blennow K, White LR, Launer LJ. CSF Abeta 42 levels correlate with amyloid-neuropathology in a population-based autopsy study. *Neurology*. Feb 25 2003;60(4):652-6.
189. Tapiola T, Alafuzoff I, Herukka SK, et al. Cerebrospinal fluid {beta}-amyloid 42 and tau proteins as biomarkers of Alzheimer-type pathologic changes in the brain. *Archives of neurology*. Mar 2009;66(3):382-9. doi:10.1001/archneurol.2008.596
190. Fagan AM, Mintun MA, Mach RH, et al. Inverse relation between in vivo amyloid imaging load and cerebrospinal fluid Abeta42 in humans. *Annals of neurology*. Mar 2006;59(3):512-9. doi:10.1002/ana.20730
191. Driscoll I, Troncoso JC, Rudow G, et al. Correspondence between in vivo (11)C-PiB-PET amyloid imaging and postmortem, region-matched assessment of plaques. *Acta neuropathologica*. Dec 2012;124(6):823-31. doi:10.1007/s00401-012-1025-1
192. Sojkova J, Driscoll I, Iacono D, et al. In vivo fibrillar beta-amyloid detected using [11C] PiB positron emission tomography and neuropathologic assessment in older adults. *Archives of neurology*. Feb 2011;68(2):232-40. doi:10.1001/archneurol.2010.357
193. Clark CM, Schneider JA, Bedell BJ, et al. Use of florbetapir-PET for imaging beta-amyloid pathology. *Jama*. Jan 19 2011;305(3):275-83. doi:10.1001/jama.2010.2008
194. Vandenberghe R, Van Laere K, Ivanoiu A, et al. 18F-flutemetamol amyloid imaging in Alzheimer disease and mild cognitive impairment: a phase 2 trial. *Annals of neurology*. Sep 2010;68(3):319-29. doi:10.1002/ana.22068
195. Peskind ER, Li G, Shofer J, et al. Age and apolipoprotein E*4 allele effects on cerebrospinal fluid beta-amyloid 42 in adults with normal cognition. *Archives of neurology*. Jul 2006;63(7):936-9. doi:10.1001/archneur.63.7.936
196. Popp J, Lewczuk P, Frommann I, et al. Cerebrospinal fluid markers for Alzheimer's disease over the lifespan: effects of age and the APOEepsilon4 genotype. *Journal of Alzheimer's disease : JAD*. 2010;22(2):459-68. doi:10.3233/jad-2010-100561
197. Kawata K, Liu CY, Merkel SF, Ramirez SH, Tierney RT, Langford D. Blood biomarkers for brain injury: What are we measuring? *Neuroscience and biobehavioral reviews*. Sep 2016;68:460-473. doi:10.1016/j.neubiorev.2016.05.009
198. Buerger K, Ewers M, Pirttila T, et al. CSF phosphorylated tau protein correlates with neocortical neurofibrillary pathology in Alzheimer's disease. *Brain : a journal of neurology*. Nov 2006;129(Pt 11):3035-41. doi:10.1093/brain/awl269

199. Hohman TJ, Dumitrescu L, Barnes LL, et al. Sex-Specific Association of Apolipoprotein E With Cerebrospinal Fluid Levels of Tau. *JAMA neurology*. Aug 1 2018;75(8):989-998. doi:10.1001/jamaneurol.2018.0821
200. Li G, Sokal I, Quinn JF, et al. CSF tau/Abeta42 ratio for increased risk of mild cognitive impairment: a follow-up study. *Neurology*. Aug 14 2007;69(7):631-9. doi:10.1212/01.wnl.0000267428.62582.aa
201. Bartel DP. MicroRNAs: target recognition and regulatory functions. *Cell*. Jan 23 2009;136(2):215-33. doi:10.1016/j.cell.2009.01.002
202. Lopez-Ramirez MA, Nicoli S. Role of miRNAs and epigenetics in neural stem cell fate determination. *Epigenetics*. Jan 2014;9(1):90-100. doi:10.4161/epi.27536
203. Saraiva C, Esteves M, Bernardino L. MicroRNA: Basic concepts and implications for regeneration and repair of neurodegenerative diseases. *Biochemical pharmacology*. Oct 1 2017;141:118-131. doi:10.1016/j.bcp.2017.07.008
204. Nagaraj S, Zoltowska KM, Laskowska-Kaszub K, Wojda U. microRNA diagnostic panel for Alzheimer's disease and epigenetic trade-off between neurodegeneration and cancer. *Ageing research reviews*. Jan 2019;49:125-143. doi:10.1016/j.arr.2018.10.008
205. Jove M, Portero-Otin M, Naudi A, Ferrer I, Pamplona R. Metabolomics of human brain aging and age-related neurodegenerative diseases. *Journal of neuropathology and experimental neurology*. Jul 2014;73(7):640-57. doi:10.1097/nen.0000000000000091
206. Cilento EM, Jin L, Stewart T, Shi M, Sheng L, Zhang J. Mass spectrometry: A platform for biomarker discovery and validation for Alzheimer's and Parkinson's diseases. *Journal of neurochemistry*. Nov 26 2018;doi:10.1111/jnc.14635
207. Quinones MP, Kaddurah-Daouk R. Metabolomics tools for identifying biomarkers for neuropsychiatric diseases. *Neurobiology of disease*. Aug 2009;35(2):165-76. doi:10.1016/j.nbd.2009.02.019
208. Schoenmaker M, de Craen AJ, de Meijer PH, et al. Evidence of genetic enrichment for exceptional survival using a family approach: the Leiden Longevity Study. *European journal of human genetics : EJHG*. Jan 2006;14(1):79-84. doi:10.1038/sj.ejhg.5201508
209. Westendorp RG, van HD, Rozing MP, et al. Nonagenarian siblings and their offspring display lower risk of mortality and morbidity than sporadic nonagenarians: The Leiden Longevity Study. *J Am Geriatr Soc*. 9/2009 2009;57(9):1634-1637. Not in File. doi:JGS2381 [pii];10.1111/j.1532-5415.2009.02381.x [doi]
210. Monaghan PJ, Lord SJ, St John A, et al. Biomarker development targeting unmet clinical needs. *Clin Chim Acta*. Sep 1 2016;460:211-9. doi:10.1016/j.cca.2016.06.037
211. Fulop T, Larbi A, Dupuis G, et al. Immunosenescence and Inflamm-Aging As Two Sides of the Same Coin: Friends or Foes? *Front Immunol*. 2017;8:1960. doi:10.3389/fimmu.2017.01960
212. Loffredo FS, Steinhauser ML, Jay SM, et al. Growth differentiation factor 11 is a circulating factor that reverses age-related cardiac hypertrophy. *Cell*. May 9 2013;153(4):828-39. doi:10.1016/j.cell.2013.04.015
213. Villeda SA, Plambeck KE, Middeldorp J, et al. Young blood reverses age-related impairments in cognitive function and synaptic plasticity in mice. *Nature medicine*. Jun 2014;20(6):659-63. doi:10.1038/nm.3569
214. Franceschi C, Bonafe M, Valensin S, et al. Inflamm-aging. An evolutionary perspective on immunosenescence. *Ann N Y Acad Sci*. Jun 2000;908:244-54.
215. Kocijan R, Muschitz C, Geiger E, et al. Circulating microRNA Signatures in Patients With Idiopathic and Postmenopausal Osteoporosis and Fragility Fractures. *J Clin Endocrinol Metab*. Nov 2016;101(11):4125-4134. doi:10.1210/jc.2016-2365