



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

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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 the advantages and limitations of potential biomarkers 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.

1. 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 (Fig. 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 (Partridge et al., 2018). 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

(Mooijaart et al., 2015). 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 (Kirkwood and Austad, 2000). 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 (Fried et al., 2011). 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

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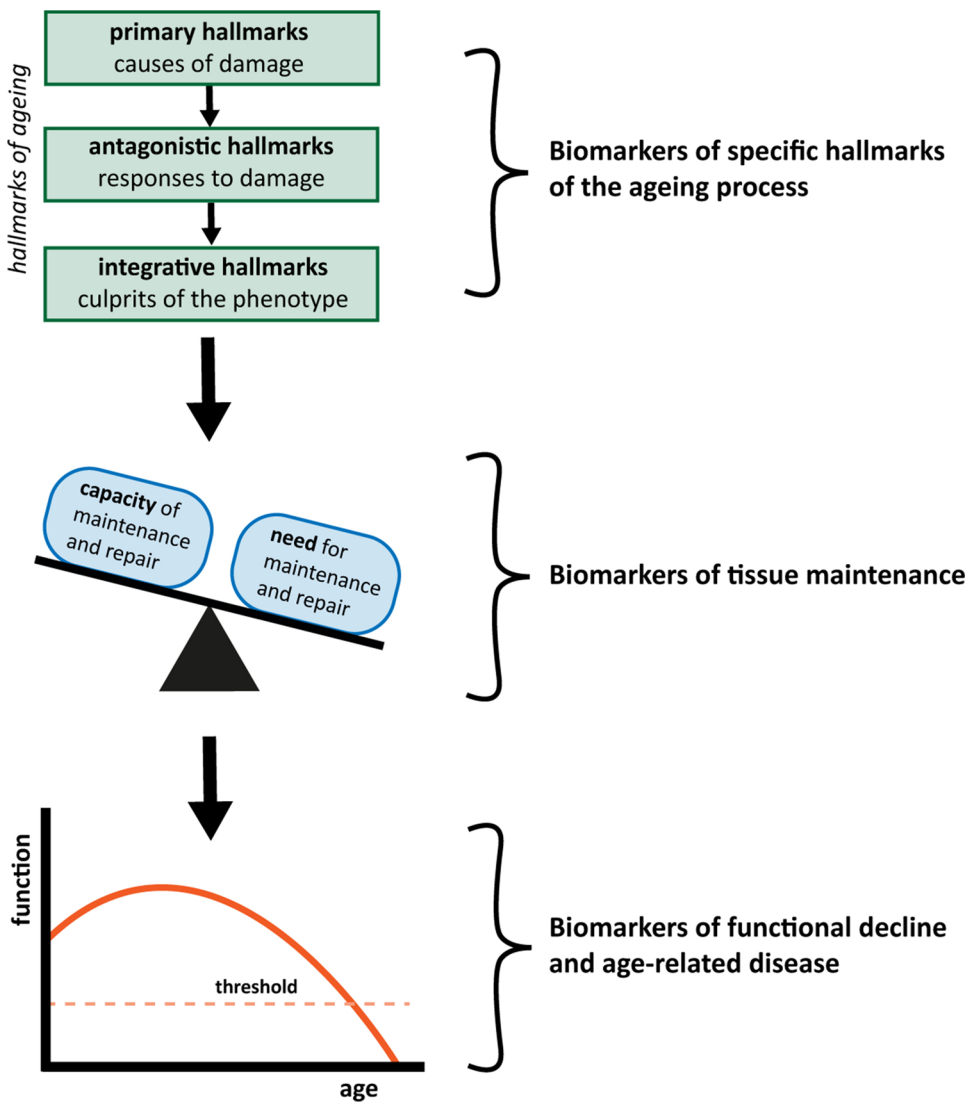


Fig. 1. Hypothesis: biomarkers of tissue maintenance are at the interface between the hallmarks of ageing and age-related functional decline and disease.

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.

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 (Strom et al., 2011). Osteoarthritis is an inflammatory process of the joints leading to damage and loss of cartilage with consequential joint pain and loss of joint mobility (Bijlsma et al., 2011), while sarcopenia is characterized by the combination of loss of skeletal muscle mass with loss of muscle strength and/or physical performance (Cruz-Jentoft et al., 2010; Fielding et al., 2011). Progressive degeneration of neurons in the brain leads to neurodegenerative diseases including Parkinson's, Alzheimer's, and Huntington's disease (Ahmad et al., 2017). 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 (Belsky et al., 2015;

Burkle et al., 2015; Lara et al., 2015; Sebastiani et al., 2017). 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 (Lopez-Otin et al., 2013). 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 (Lindahl, 2008). 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 Fig. 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.

2. 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” (WHO, 2001). 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 (Belsky et al., 2015; Burkle et al., 2015; Lara et al., 2015; Sebastiani et al., 2017). 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 (Cooper et al., 2010; Koopman et al., 2015; Lara et al.,

2015; Studenski et al., 2011; Takata et al., 2014). 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 (Lopez-Otin et al., 2013). 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 (Khan et al., 2017). 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 (Bai, 2015). Also leukocyte telomere length is a potential biomarker of ageing, but the clinical evidence is yet inconclusive (Sanders and Newman, 2013). 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 (Horvath and Raj, 2018; Jylhava et al., 2017). 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 (Burkle et al., 2015). 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 (Longo and Finch, 2003). 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 (Trout et al., 2007). Mitochondrial dysfunction results in the production of reactive oxygen species (ROS) which causes cellular damage (Hekimi et al., 2011). 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 (SAHF) and γ H2AX (DNA damage) foci, and senescent cells exhibit a senescence associated secretory phenotype (SASP) (Munoz-Espin and Serrano, 2014; Sharpless and Sherr, 2015). 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 (Mosaad, 2014). Besides increases in immunosenescence and inflammaging, features of altered intercellular

communication comprise the decline in sex hormones with age, including estrogen and testosterone (Kaufman and Vermeulen, 2005; Melmed et al., 2016). 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.

3. 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 (Melmed et al., 2016). 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 (Boron and Boulpaep, 2009). Mechanical loading is important for maintaining bone mass and studies have shown that both bone formation and resorption markers are increased after physical activity (Ardawi et al., 2012).

3.1. 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 (Strom et al., 2011). Besides age and being postmenopausal, other risk factors for low bone mineral density (BMD) are a family history of osteoporosis, cigarette smoking, low physical activity, low body mass index, low calcium intake, low vitamin D level, and use of corticosteroids (Curtis et al., 2015). 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 (NIH Consensus Development Panel, 2001; Rachner et al., 2011). 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 (Kannegaard et al., 2010). 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 (Strom et al., 2011).

3.2. Biomarkers of bone tissue turnover

Bone turnover markers have been identified and reviewed by others (Garnero, 2017; Wheeler et al., 2013; Woitge and Seibel, 2017). 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 helical peptide (HELPE), 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 (Vasikaran et al., 2011). 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 Fig. 2.

3.2.1. 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 (Chubb, 2012). CTX-I is relatively stable at room temperature, especially in EDTA (Stokes et al., 2011). 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 (Ardawi et al., 2010; Jenkins et al., 2013; Michelsen et al., 2013). CTX-I shows a circadian rhythm with its nadir in the afternoon around 14:00 h and its maximum in the night around 5:00 h (Dovio et al., 2008; Wichers et al., 1999). 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 (Bjarnason et al., 2002; Christgau et al., 2000; Henriksen et al., 2003; Qvist et al., 2002). 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 (Qvist et al., 2002; van der Spoel et al., 2019). Generally, biomarkers of bone resorption show a stronger circadian rhythm than biomarkers of bone formation (Garnero, 2017; Wheeler et al., 2013).

3.2.2. 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 intraindividual variability, a good assay precision, and is stable at room temperature (Redmond et al., 2016; Stokes et al., 2011; Swanson et al., 2017; Vasikaran et al., 2011; Wolthers et al., 2001). Studies on the 24-h rhythm of PINP are conflicting. Two studies did not observe a 24-h rhythm, while two other studies found somewhat higher levels in the night, albeit in men only in one of the two studies (Redmond et al., 2016; Swanson et al., 2017; van der Spoel et al., 2019; Wolthers et al., 2001). 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 (Jenkins et al.,

2013; Michelsen et al., 2013). 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 (Clowes et al., 2002; Xiang et al., 2016; Yavropoulou et al., 2011).

3.2.3. 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 (Lombardi et al., 2015). 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 stage 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 (Ardawi et al., 2010; Hannemann et al., 2013; Midtby et al., 2001). 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 (Eastell et al., 2001; Greenspan et al., 1997; Gundberg et al., 1985; Pedersen et al., 1995; Pietschmann et al., 1990).

3.2.4. 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 (Dallas et al., 2013). 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 (Dallas et al., 2013). 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 (Bafico et al., 2001; Li et al., 2005). Serum sclerostin levels were higher in men than in women and levels correlated positively with age (Amrein et al., 2012; Ardawi et al., 2011; Bhattoa et al., 2013; Dovjak et al., 2017; Modder et al., 2011). No circadian rhythm of sclerostin has been observed, although sclerostin levels varied over 24 h in one study (Swanson et al., 2017; van der Spoel et al., 2019). DKK1 levels were somewhat higher in older individuals than in younger individuals and levels were higher in female than in male geriatric patients (Bradburn et al., 2016; Dovjak et al., 2017). 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 (van der Spoel et al., 2019). 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.

3.3. 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 (Gordon and Hahn, 2010), while the proteoglycans bind water through their negative charge, conferring flexibility and shock-absorbing properties (Chandran and Horkay, 2012), both vital to cartilage function. Turnover rate of articular cartilage ECM is generally low but differs dramatically between its components; proteoglycans have an estimated half-life of 25 years (Maroudas et al., 1998), while the estimated half-life of collagen in cartilage equals 117 years (Verzijl et al., 2000).

3.4. 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 (Sharma et al., 2013). Patients often experience joint pain and stiffness, and in severe disease limitation of movement (Bijlsma et al., 2011). 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 (Kellgren and Lawrence, 1957), 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 (Bijlsma et al., 2011). 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 (Wittenauer et al., 2013). The established risk factors for osteoarthritis include obesity, high age and female sex, whereas the respective roles of smoking, bone density and dietary factors are still under debate (Allen and Golightly, 2015). 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 advice, and in late stage of disease surgical joint replacement (Bijlsma et al., 2011), which is associated with high perioperative morbidity and persistent functional impairment after 12 months (Walsh et al., 1998). 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 presymptomatic 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.

3.5. 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 (Charni-Ben Tabassi and Garnero, 2007; Woitge and Seibel, 2017). 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 PINP) (Charni-Ben Tabassi and Garnero, 2007; Garvican et al., 2010b; Woitge and Seibel, 2017). The

main non-collagenous marker of cartilage turnover is cartilage oligomeric matrix protein (COMP), a glycoprotein constituent of articular cartilage (Garvican et al., 2010a; Woitge and Seibel, 2017). 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 (Woitge and Seibel, 2017). The main proposed biomarkers of cartilage turnover are listed in Fig. 2.

3.5.1. 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 (Posey et al., 2018). COMP interacts with several other ECM proteins, aiding matrix assembly and stability through binding collagen fibres (Acharya et al., 2014). Upon degradation of cartilage, COMP is released into the synovial fluid and consequently leaks into the circulation (Neidhart et al., 1997). Although COMP was first discovered in cartilage, it is expressed in multiple other tissues including tendons, synovium, cardiomyocytes and activated platelets (Posey et al., 2018). Nevertheless, COMP is one of the most robust biomarkers for osteoarthritis (Lotz et al., 2014). Measurement of COMP can occur in serum or plasma (Tufan et al., 2007) and in synovial fluid (Saxne and Heinegard, 1992), though collection of synovial fluid in healthy individuals proved difficult to perform (Hyldahl et al., 2016). Physical activity was found to increase circulating COMP levels acutely (Hyldahl et al., 2016; Mundermann et al., 2005; Niehoff et al., 2011), and a second peak occurs five hours after exercise (Mundermann et al., 2005). However, the effect of exercise is not as pronounced in individuals who exercise habitually (Cattano et al., 2017; Vuolteenaho et al., 2014). On the contrary, bed rest decreases circulating levels of COMP (Liphardt et al., 2018; Mundermann et al., 2005), though serum levels normalize within one day after discontinuation of bed rest (Liphardt et al., 2018). Because of its sensitivity to physical activity, most studies include a 24–36 h limitation of exercise prior to the test day (Mundermann et al., 2005; Niehoff et al., 2011) and a short resting period of 15–30 min immediately before drawing blood for COMP measurements to reduce effects of activities of daily living on circulating COMP levels (Cattano et al., 2017; Mundermann et al., 2005; Niehoff et al., 2011).

3.5.2. 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 (Charni-Ben Tabassi et al., 2008). 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 (Christgau et al., 2001). Measurement can be performed in plasma (Brayda-Bruno et al., 2017), urine (Christgau et al., 2001), serum and synovial fluid (Lohmander et al., 2003). 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 (Christgau et al., 2001). Generally, uCTX-II levels are lower in older than in young adults (Mouritzen et al., 2003; Rotterud et al., 2014). Individuals with a higher BMI tend to have higher uCTX-II than lean individuals (Holinka et al., 2008; Karsdal et al., 2010; Mouritzen et al., 2003). Levels of uCTX-II are higher in women than in men (Karsdal et al., 2010; Mouritzen et al., 2003). Estrogen levels appear to influence uCTX-II, since the highest levels are observed in postmenopausal women (Bay-Jensen et al., 2009; Mouritzen et al., 2003) and a decrease of uCTX-II was described with the use hormonal replacement therapy (Ausmanas et al., 2007; Mouritzen et al., 2003) and selective estrogen receptor modulator (Christgau et al., 2004). Bisphosphonate use was shown to

decrease uCTX-II in postmenopausal women, with return to baseline levels after withdrawal of bisphosphonate treatment (Corsetti et al., 2015). 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 (Karsdal et al., 2010), while no rhythmicity was observed in an earlier study (Christgau et al., 2001). 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 (Brayda-Bruno et al., 2017).

3.5.3. 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 (Peltonen et al., 1985). 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 (Olsen et al., 2007). However, type 2 procollagen is synthesized in two variants due to alternative splicing, resulting in procollagen type 2A and type 2B (Ryan and Sandell, 1990). The splicing is cell type specific: procollagen 2A appears predominantly in chondroprogenitor cells, whereas type 2B is mainly produced by mature chondrocytes (Sandell et al., 1991). Both splice variants have distinct N-terminal propeptides; PIINP and PIIBNP respectively. In osteoarthritis patients, PIINP was present in deep zone cartilage (Bay-Jensen et al., 2008) and higher circulating levels of PIINP were associated with slower progression of disease (Berry et al., 2010). However, transcription of procollagen type 2A was only seen in osteoarthritic cartilage and not in healthy cartilage tissue (Aigner et al., 1999). Therefore, PIINP may not be a suitable marker 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 (Gudmann et al., 2014), and has recently been used to distinguish arthropathies (Gudmann et al., 2016; Hua et al., 2017) and for early drug development (Kjelgaard-Petersen et al., 2019; Reker et al., 2017). Even though further validation is still warranted, PIIBNP is a promising biomarker for collagen type 2 synthesis in healthy adult populations.

3.6. 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 (Boron and Boulpaep, 2009). 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 (Evans, 2010). 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 (Ferrando et al., 1996; Paddon-Jones et al., 2006).

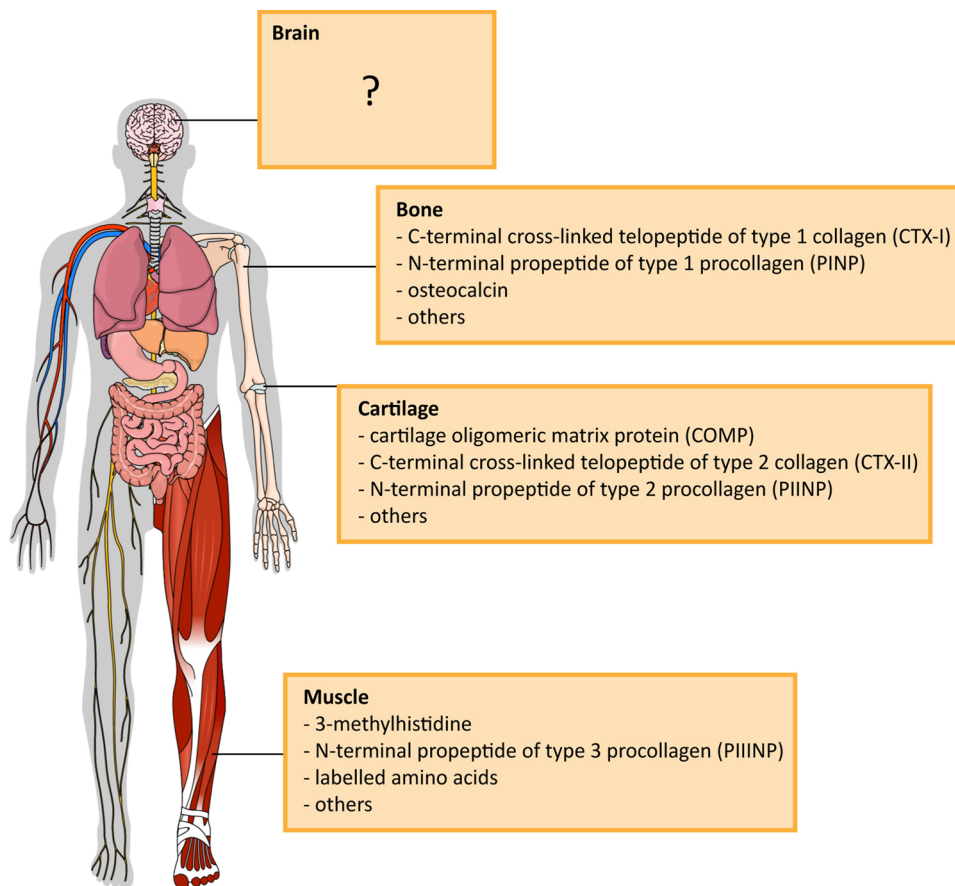


Fig. 2. Overview of proposed biomarkers of tissue maintenance.

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.

3.7. 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 (Cruz-Jentoft et al., 2010; Fielding et al., 2011). 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 (Curtis et al., 2015; Fielding et al., 2011). Sarcopenia can be diagnosed when a patient's muscle mass is less than 20th percentile of values for healthy young adults, determined by 24-h urinary creatinine (except in patients with renal insufficiency), or more indirect by bioelectrical impedance, dual-energy X-ray absorptiometry (DEXA) or other 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 (Cruz-Jentoft et al., 2010). Sarcopenia biomarkers have been reviewed elsewhere (Kalinkovich and Livshits, 2015). Sarcopenia is a strong predictor of late-life disability (Doherty, 2003; Janssen et al., 2002). 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 (Shafiee et al., 2017). 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 (Fiatarone et al., 1994).

3.8. 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) (Cesari et al., 2012; Schaap et al., 2006). Muscle-specific biomarkers are serum myostatin, which is a suppressor of muscle growth (Lee, 2004), 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 (Brancaccio et al., 2007). An overview of the main proposed biomarkers of muscle protein synthesis and breakdown is given in Fig. 2.

3.8.1. 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 (Long et al., 1975; Sugden and Fuller, 1991). 3-Methylhistidine is preferably measured in (24-h) 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 (Sheffield-Moore et al., 2014). Levels of 3-methylhistidine were not responsive to exercise (Calles-Escandon et al., 1984; Hansen et al., 2009).

3.8.2. PIINP

A biomarker of skeletal muscle remodeling is plasma N-terminal propeptide of type 3 procollagen (PIIINP) (Cesari et al., 2012; Curcio et al., 2016). PIIINP 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 (Niemela, 1985; Niemela et al., 1985; Prockop et al., 1979). Consequently, PIIINP can be measured in serum with precision and accuracy. PIIINP levels are shown to respond to exercise, testosterone, and growth hormone (GH) (Bhasin et al., 2009; Chen et al., 2011; Crameri et al., 2004; Powrie et al., 2007; Wallace et al., 2000).

3.8.3. Infusion of isotopically labelled amino acids

Another method to determine the rate of protein turnover in muscle is by infusion of isotopes (Atherton et al., 2015; Rennie et al., 1994). 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.

3.9. 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 (Altman and Das, 1965), and later in the subventricular zone (Lois and Alvarez-Buylla, 1993) from which the new neurons also migrate to other areas of the brain (Lenington et al., 2003). However, in human brains the presence of adult neurogenesis is still under debate (Bergmann et al., 2015; Sorrells et al., 2018). 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.

3.10. Neurodegenerative disease

The hallmark of neurodegenerative diseases is progressive loss of neurons, accompanied by gradual development of cognitive impairments and motor disorders (Ahmad et al., 2017). 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 (Hardy and Selkoe, 2002), neurofibrillary tangles (Ballatore et al., 2007) and Lewy body inclusions (Arnaoutoglou et al., 2019). 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 (Pini et al., 2016), while in Parkinson's disease the substantia nigra and the basal ganglia are predominantly affected (Zeighami et al., 2019). 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 (Cholerton et al., 2016; Pietzuch et al., 2019). 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 (Ahmad et al., 2017). 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 (WHO, 2017). Other risk factors include sedentary lifestyle, obesity, tobacco smoking and hypertension (Livingston et al., 2017). Currently no disease modifying therapies exist for neurodegenerative diseases, despite decades of pharmaceutical research (Cummings et al., 2019). 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.

3.11. Biomarkers of brain tissue maintenance

Research into early markers of neurodegenerative diseases has been conducted extensively, especially for Alzheimer's dementia (Molinuevo et al., 2018). 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) (Stappert et al., 2018), natriuretic peptides (NPs) (Mahinrad et al., 2016), brain-derived neurotrophic factor (BDNF) (Numakawa et al., 2018), and glial cell line-derived neurotrophic factor (GDNF) (Airaksinen and Saarma, 2002; Kim and Kim, 2018). The predictive markers can be grouped to three categories; they are related to accumulation of misfolded proteins, progressive brain atrophy or metabolism. Accumulation of amyloid β_{42} ($A\beta_{42}$) and tau can be observed before brain atrophy occurs (Jack et al., 2013), and can be considered an early marker of disturbed tissue homeostasis. After prolonged net neurodegeneration, brain atrophy can be visualized using different imaging techniques (Dickerson and Wolk, 2012). For metabolism several metabolomic biomarkers have been associated with dementia risk, of which especially compounds from lipid metabolism appear to correlate with risk of dementia (Mapstone et al., 2014).

3.11.1. 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 (DeCarli et al., 2007; Grundman et al., 2002), while in early stage Parkinson's disease atrophy of the substantia nigra and basal ganglia are the primary abnormalities (Zeighami et al., 2019). More general anatomical abnormalities include signs of cerebral small vessel disease and loss of cortical thickness (Dickerson and Wolk, 2012; Zwanenburg and van Osch, 2017). Although the resolution of MRI scans has improved dramatically over the past decades from 0.5 T to 7 T (Zwanenburg et al., 2013), 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 (Gallivanone et al., 2016). 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 (Bonte et al., 2017; Sarikaya et al., 2018). 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 (Cholerton et al., 2016). 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.

3.11.2. $A\beta_{42}$ 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 $A\beta$ and neurofibrillary tangles are among the earliest abnormalities in neurodegenerative processes (Jack et al., 2013). It is hypothesized that these accumulated proteins are neurotoxic, especially oligomerization appears to contribute to neurotoxic effects of $A\beta_{42}$ and tau (Ballatore et al., 2007; Hardy and Selkoe, 2002).

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 (Klunk et al., 2004; Olsson et al., 2016). Counterintuitively, higher plaque burden of amyloid deposition on autopsy was associated with lower concentrations of $A\beta_{42}$ in the CSF (Strozyk et al., 2003; Tapiola et al., 2009). 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 (Fagan et al., 2006). Cerebral burden of amyloid β can also be measured using PET. The first method developed is Pittsburgh Compound-B (PiB) PET (Klunk et al., 2004). PiB PET imaging is consistent with post-mortem amyloid β plaque burden (Driscoll et al., 2012; Sojkova et al., 2011). Since PiB is technically difficult to work with due to its short half-life of only 20 min, alternative ligands have been developed with similar imaging properties (Clark et al., 2011; Vandenberghe et al., 2010). For all measurements related to amyloid β , it is important to realize that there is a strong influence of age and ApoE $\epsilon 4$ genotype (Peskind et al., 2006; Popp et al., 2010).

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 (Kawata et al., 2016), phosphorylated tau (p-tau) is thought to be more specific for neurofibrillary tangles (Buerger et al., 2006). Nevertheless, higher CSF concentrations of both total tau (t-tau) and p-tau were associated with more neurofibrillary pathologic abnormalities on autopsy (Buerger et al., 2006; Tapiola et al., 2009). 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 (Olsson et al., 2016). For measurements of tau the factors of influence are not fully clarified, though ApoE $\epsilon 4$ genotype and possibly age and sex appear to associate with higher tau concentrations (Hohman et al., 2018; Li et al., 2007).

3.11.3. Other (potential) biomarkers of brain tissue maintenance

Although measures of atrophy and cerebral accumulation of misfolded proteins have been investigated extensively, these 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 (Bartel, 2009). Several miRNAs appear to spatially and temporarily regulate replication and differentiation of neurons (Lopez-Ramirez and Nicoli, 2014; Saraiva et al., 2017; Stappert et al., 2018). However, validation of identified miRNA signals is scarce and many studies have shown discordant results (Nagaraj et al., 2019). Other potentially interesting regulatory molecules could be NPs, which were shown to play an

essential role in fluid homeostasis and neuro-inflammation (Mahinrad et al., 2016). BDNF could also be of interest as a biomarker, though previous research results have been conflicting (Numakawa et al., 2018). 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 (Jove et al., 2014). 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 (Cilento et al., 2018; Jove et al., 2014; Quinones and Kaddurah-Daouk, 2009). 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 (Jove et al., 2014).

4. 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. Fig. 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

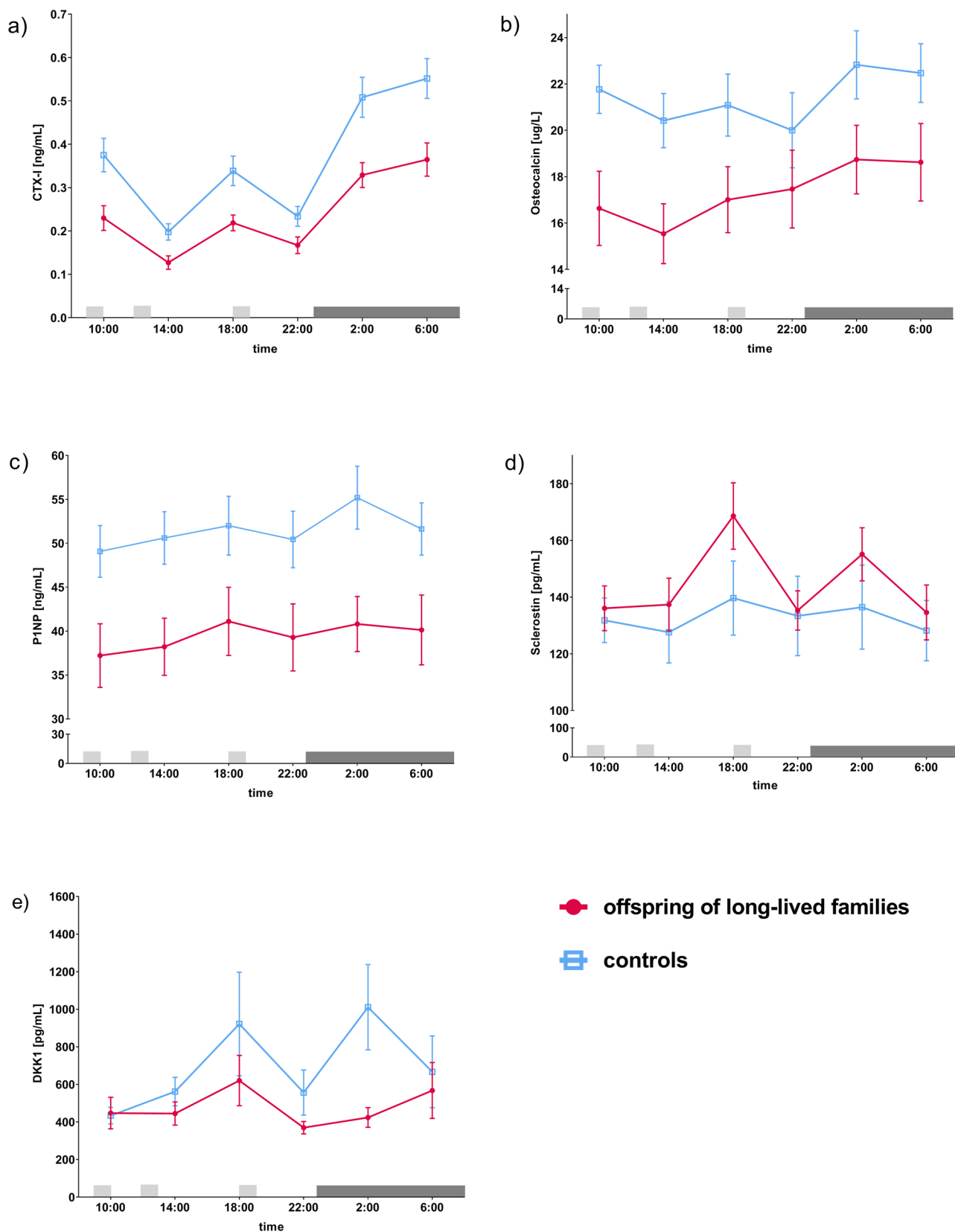


Fig. 3. Bone markers over 24 h stratified for offspring of long-lived families and controls.

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 h 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.

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 (Schoenmaker et al., 2006). 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 (Westendorp et al., 2009). 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 h (van der Spoel et al., 2019). In Fig. 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 (Burkle et al., 2015). However, before validated biomarkers can be broadly used in research or the clinic, measurement of these biomarkers should be standardized (Monaghan et al., 2016).

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 (Fulop et al., 2017; Munoz-Espin and Serrano, 2014). 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 (Loffredo et al., 2013; Villeda et al., 2014). 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 (Franceschi et al., 2000). 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 (Kocijan et al., 2016). Therefore, future research could besides validating proposed biomarkers of tissue maintenance, include identification of circulating factors crucial for tissue maintenance.

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