Review

The Effect of Aging and Mechanical Loading on the Metabolism of Articular Cartilage

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ABSTRACT. ***Objective.*** The morphology of articular cartilage (AC) enables painless movement. Aging and mechanical loading are believed to influence development of osteoarthritis (OA), yet the connection remains unclear.

Methods. This narrative review describes the current knowledge regarding this area, with the literature search made on PubMed using appropriate keywords regarding AC, age, and mechanical loading.

Results. Following skeletal maturation, chondrocyte numbers decline while increasing senescence occurs. Lower cartilage turnover causes diminished maintenance capacity, which produces accumulation of fibrillar crosslinks by advanced glycation end products, resulting in increased stiffness and thereby destruction susceptibility.

Conclusion. Mechanical loading changes proteoglycan content. Moderate mechanical loading causes hypertrophy and reduced mechanical loading causes atrophy. Overloading produces collagen network damage and proteoglycan loss, leading to irreversible cartilage destruction because of lack of regenerative capacity. Catabolic pathways involve inflammation and the transcription factor nuclear factor-κB. Thus, age seems to be a predisposing factor for OA, with mechanical overload being the likely triggering cause. (J Rheumatol First Release March 1 2017; doi:10.3899/jrheum.160226)

Key Indexing Terms:
AGE OSTEOARTHRITIS BIOMECHANICS CARTILAGE CYTOKINES

Articular cartilage (AC) covers bone surfaces and allows for almost friction-free movement. Unfortunately, AC is susceptible to acute injury and degenerative conditions, e.g., osteoarthritis (OA), and because cartilage has very poor healing potential, OA is a considerable medical challenge. OA is no longer solely seen as 1 single disease, instead 5 OA phenotypes have been suggested, i.e., genetic, metabolic, pain, age, and structural/post-traumatic[1]. Our narrative review is meant as a covering overview of the main OA phenotypes (related to aging and mechanical loading), and is aimed to include studies of molecular, biochemical, physiological, and clinical designs. To clarify these OA phenotypes, basic information about AC morphology and key components is provided. This is followed by a review of the effect of age and mechanical influence on the morphology, along with the underlying cell signaling, because, as demonstrated, OA is not merely a mechanical/physical "wear and tear" disease. The literature search was performed on PubMed using appropriate keywords regarding exercise/mechanical load, articular cartilage, metabolism/turnover, OA, extracellular matrix, and cell signaling/transduction.

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Morphology

AC consists of the chondrocyte surrounded by an extracellular matrix (ECM), subdivided into areas in a pericellular matrix (PCM) immediately adjacent to the cell, a territorial matrix farther away, and an interterritorial matrix[2]. ECM contains a fibrillar network of both collagens and noncollagenous matrix components embedded in a viscous gel-like ground/basic substance. The fibers are oriented differently and divide the uncalcified AC into 3 zones: superficial zone (SZ) with parallel fiber orientation, intermediate zone (IZ) with random, and finally deep zone (DZ) with vertical orientation. A tidemark represents the DZ transition into the mineralized/calcified fourth zone followed by the subchondral bone below[3]. The ground/basic substance contains the extra-

cellular fluid and large proteoglycan aggregates built from a single hyaluronan/hyaluronic acid (HA) backbone with some 100 proteoglycans (PG) attached[2]. Aggrecan contains 3 globular domains (G1–G3). G1 binds noncovalently to hyaluronan stabilized by link protein (Figure 1). G3 is able to bind to matrix proteins, while G2's function is unknown[2]. Aggrecan is the major PG in AC, built from a core protein attached with glycosaminoglycans (GAG), mainly chondroitin sulphate (CS) and keratan sulphate (KS), and oligosaccharide chains[4]. The GAG attract cations and water, resulting in swelling, and are counteracted by the fibrillar network through enzymatic crosslinks, which provide tensile strength and low compliance[5]. With joint loading, the proteoglycan aggregates are compressed and distribute the force onto the joint surface, thereby reducing the pressure on the AC[6]. The joint surface is lined/coated with the glycoprotein lubricin/proteoglycan–4 and HA made from both chondrocytes and synoviocytes, which reduce mechanical friction considerably[7], thus enabling AC to distribute and transfer force between bones seamlessly.

The influence of aging on articular cartilage

Effect of aging on chondrocytes. Mesenchymal stem cells differentiate into chondrogenic progenitor cells, and after the placement of AC, the chondrocytes remain in a post-mitotic stage[8]. However, chondrogenic progenitor cells in SZ can migrate to a damaged area, proliferate, and cover this with a continuous sheet and lubricin coating[9]. Why, then, does AC exhibit such poor healing capacity? With increasing age, more chondrocytes are found in the state of senescence shown by both diminished mitotic activity and telomere length[10,11]. Diminished telomere length occurs naturally following replications, and by chronic overloading or following a trauma, the chondrocytes undergo proliferation, thereby providing a link between mechanical loading and senescence[11].

Exposure of human cell cultures to considerable amounts of mechanical load lead to increased oxidative stress and senescence without a diminished telomere length[10]. This oxidative stress induces damage to the mitochondria, leading to either senescence or apoptosis, both limiting the functional lifespan of chondrocytes[11]. Cumulated oxidative stress increases with age[12], while the number of chondrocytes drops proportionally[13], most profoundly in SZ, possibly because of lubricin loss[7]. Because chondrocytes are in post-mitotic state, it seems unlikely that the intrinsic/replicative–induced senescence is involved, while the extrinsic/stress–induced seems paramount. With age, the remaining chondrocytes have less ability to synthesize matrix components in response to growth factors, but increase their inflammatory cytokine response[14] because of changed sensitivity.

Aging and collagen turnover. The biomechanical stiffness of the collagen network increases with age unrelated to normal enzymatic crosslinking[5], but instead proportionally to nonenzymatic crosslinks by advanced glycation end products (AGE)[15]. Magnetic resonance imaging (MRI) shows that AC in healthy elderly individuals (50–78 yrs) has a diminished capacity for deformation *in vivo* when compared with younger individuals (20–30 yrs), likely resulting from this increased content of AGE crosslinks and collagen stiffness[16]. Stiff biomechanics that are nonenzymatic from AGE or enzymatic through extracellular lysyl oxidase have been shown to stimulate cartilage destruction through the Rho-Rho kinase-myosin light chain pathway (Figure 2)[17]. Further, receptors for AGE found on the chondrocyte with increasing age and OA lead to the activation of the transcription factor nuclear factor-κB (NF-κB; p50/p65) and the synthesis of matrix breakdown enzymes (Figure 2)[18].

The content of pentosidine, a known fluorescent AGE, rises linearly after the age of 20 but is undetectable before, indicating that collagen only undergoes significant breakdown and renewal in the first 2 decades[5]. The collagen

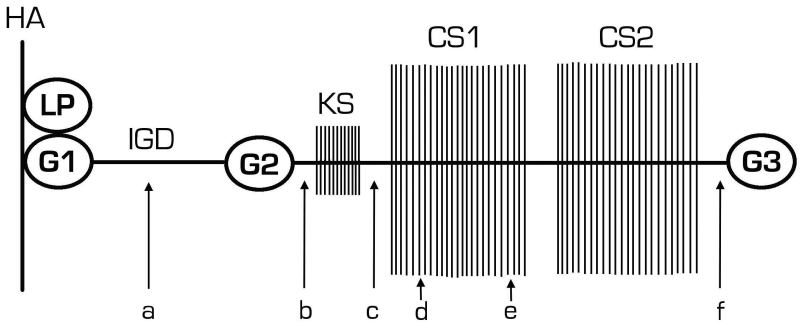

Figure 1. Schematic illustration of the HA backbone onto which aggrecan attaches with its first globular domain (G1) under the stabilizing influence of LP. Aggrecan contains 3 globular domains (G1–G3), an IGD, a KS-rich domain, and 2 CS-rich domains (CS1 and CS2). Matrix metalloproteinases cleave aggrecan in various regions or sites (a–f), yielding different breakdown products. ADAMTS cleave in the "a-site," "f-site," and 4 other sites in the CS2 region. HA: hyaluronic acid; LP: link protein; IGD: interglobular domain; KS: keratan sulphate; CS: chondroitin sulphate.

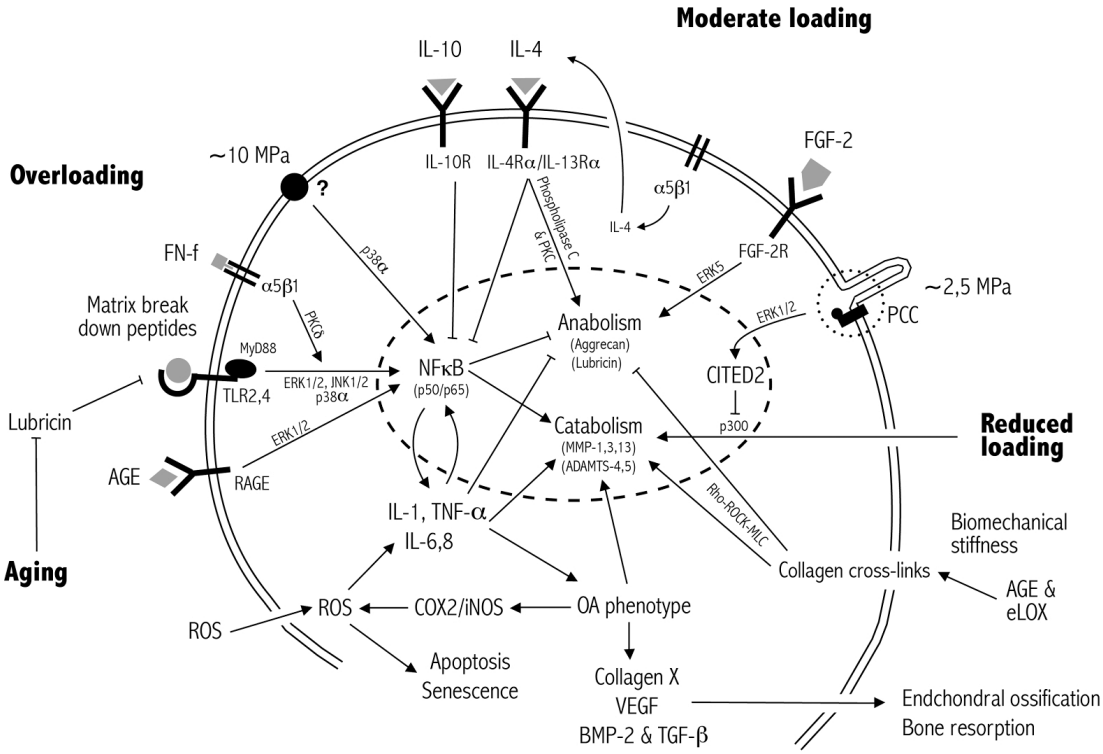

Figure 2. Simplified overview showing a chondrocyte with various receptors in the cell membrane (double black line) and their downstream effect on processes in the nucleus (broken line). Moderate loading leads to increased activity of FGF-2, IL-4, IL-10, which suppress NF-κB and its catabolic action. These factors also lead to an anabolic response with increased synthesis of aggrecan. Further, a primary cilia along with its other receptors and factors form a complex (PCC; dotted line), which inhibits the catabolism mediated by unloading. Overloading results in the destruction of matrix components leading to the formation of breakdown products (peptides) from FN-f, fibromodulin, decorin, collagen, and COMP that activate integrin receptors as well as TLR. This increases NF-κB action and leads to a catabolic response with synthesis of MMP and aggrecanases (ADAMTS). Biomechanical stiffness leads to activation of the Rho-ROCK-MLC, which inhibits anabolism and promotes catabolism. With increasing age, lubricin expression decreases along with its inhibitory effect on the TLR. Further, increased reactive oxygen species are seen, as well as the accumulation of AGE, which activate RAGE, yet again leading to an NF-κB–mediated catabolic response. NF-κB induces expression of proinflammatory mediators IL-1, IL-6, IL-8, and TNF-α, resulting in an osteoarthritis phenotype with increasing levels of COX-2, iNOS, and thereby PGE2 and NO, as well as collagen type X, VEGF, BMP-2, and TGF-β. This leads to the formation of osteophytes and subchondral changes. FGF-2: fibroblast growth factor 2; IL-4: interleukin 4; NF-κB: nuclear factor-κB; PCC: primary cilia complex; FN-f: fibronectin fragments; COMP: cartilage oligomeric protein; TLR: Toll-like receptors; MMP: matrix metalloproteinases; Rho-ROCK-MLC: Rho-Rho kinase-myosin light chain; AGE: advanced glycation end products; RAGE: receptors for AGE; TNF-α: tumor necrosis factor-α; COX-2: cyclooxygenase 2; iNOS: inducible nitric oxide synthase; PGE2: prostaglandin E2; NO: nitric oxide; VEGF: vascular endothelial growth factor; BMP-2: bone morphogenetic protein 2; TGF-β: transforming growth factor-β; CITED2: CBP/p300-interacting transactivator with ED-rich tail 2; eLOX: extracellular lysyl oxidase; ERK: extracellular signal–regulated kinase; JNK: c-Jun N-terminal kinase; MyD88: myeloid differentiation factor 88; PKC: protein kinase C; ROS: reactive oxygen species.

turnover was estimated to a half-life of 200–400 years using a racemization approach dependent on temperature, protein structure, and pH[19], which therefore may not be conclusive. The carbon-14 bomb-pulse method uses the atmospheric rise in carbon-14 created by earlier nuclear bomb testing, and has shown negligible collagen turnover after adolescence in humans regardless of either disease (OA) or damage[20]. However, any definitive studies in humans regarding collagen renewal in cartilage remain to be performed.

Aging and aggrecan metabolism. The 2 major enzyme families cleaving aggrecan are the matrix metalloproteinases (MMP) and the real aggrecanases (ADAMTS)[21]. They have different preferential cleaving sites and produce different fragments or pieces of aggrecan called neoepitopes (Figure 1). These remain attached to the matrix, float around the ECM unattached, or are lost into the synovial fluid by diffusion[22]. Aggrecan cleaving in the "a-site" and "f-site" is particularly interesting because this leads to complete detachment, leaving only the G1 and part of the interglobular domain (IGD) attached[22]. In humans, "f-site" cleavage is the initial event[21]. The IGD contains cleaving sites for MMP and ADAMTS, and it has been proposed that ADAMTS cleave

IGD in full-length aggrecan, while MMP do not[23]. Others have shown that cleavage by MMP happens in a particular order, namely at G3, then both at IGD and between CS1 and CS2 region, then in CS1, between CS1, and KS region, and finally between KS region and G2 (Figure 1)[21]. This cleaving sequence will lead to diminished amounts of CS because of the elimination of CS2 followed by the CS1 region. The finding of a relatively larger ratio of KS compared with CS with increasing age supports this theory[24]. In contrast, MMP-induced neoepitopes remain in the matrix, whereas neoepitopes made by ADAMTS do not[21]. Because breakdown by both MMP and ADAMTS releases both G1 and G3, it can seem unclear why only cleaving by ADAMTS leads to loss, especially when the resulting neoepitopes are larger with both the CS1 and KS regions intact. The difference, then, must lie in the IGD, which is slightly larger when cleaved by MMP, and the N- and O-bound side chains located there must function as linkage[21]. Thus, aggrecan cleaving by MMP seems beneficial as part of the normal turnover, while ADAMTS results in AC loss[21,23]. Healthy AC contains a larger amount of MMP-induced neoepitopes with increasing age and supports this conclusion[25].

Both PG synthesis and breakdown decrease with increasing age, while pentosidine content rises[26]. AGE has been a suggested cause. It is speculated that it prevents breakdown enzymes from access either by a conformational change or by steric means[26]. However, to our knowledge, this has not been proven; rather it might be that AGE accumulation is actually caused by the reduced PG turnover possibly due to senescence. Regardless of the connection to AGE accumulation, it seems clear that PG turnover is decreased as aging occurs. Full-size aggrecan half-life was estimated at 3.2 years with the complete breakdown at 23.5 years[27]. This supports previous results showing that cleavage of the C-terminal is an early event leading to the accumulation of G1 in AC as aging occurs[24].

Aging results in senescence with changed chondrocyte metabolism and reduced turnover leading to mechanical stiffness and thus, susceptibility for damage.

The influence of mechanical loading on AC

Effect of reduced mechanical load on AC composition. In paraplegic patients, MRI of the knees have shown diminished AC thickness between 9% and 13% following 1 year of reduced load[28], and partial loading following an ankle fracture leads to thinning of the AC in the knee of the affected leg[29]. Animal models provide further details, where knee joint immobilization in dogs leads to reduced GAG content, especially from SZ, unaltered collagen content, and AC softening[30].

Thus, lack of mechanical stimulation results in thinner and softer AC. This might make the AC more susceptible to traumas because collagen damage occurs earlier in thin cartilage[31]. Further, *in vitro* studies suggest that collagen fibrils are more prone to degradation when in a slack state compared with under tension[32].

Immobilization has been proven to yield increasing amounts of MMP-1[33] and MMP-3, with MMP-3 being necessary for the increase in ADAMTS-5 levels also seen with immobilization[34]. The medial tibial cartilage has the main loss of proteoglycan aggregates in SZ[34], thereby keeping in accordance with the morphological changes described in animals. In rats, unloaded passive movements were preventive of this immobilization-derived cartilage atrophy mediated by MMP and ADAMTS-5[33,34]. This rise in breakdown enzymes during unloading could occur because of lack of expression of cAMP-responsive element-binding protein/p300-interacting transactivator with ED-rich tail 2 (CITED2), which inhibits the coactivator p300 leading to synthesis of MMP[35]. CITED2 expression results from the strain activation of a primary cilia[33], with several signaling processes including the mitogen-activated protein kinase (MAPK) extracellular signal-regulated kinase (ERK1/2; Figure 2)[35]. Another factor could be a lack of interleukin (IL)-4 or IL-10, which hinders their suppressive effect on NF-κB, thus resulting in MMP-induced catabolism (Figure 2).

In dogs, remobilization does not fully restore the stiffness or the GAG content in the areas of the joint without weight-bearing[30]. Thus, the atrophy only appears partially reversible and prevention thereby seems the best strategy.

Effect of moderate mechanical loading on AC composition. In dogs, moderate running showed increased PG content in DZ[36]. In humans, an MRI study on 18 adults showed no difference in AC thickness between lifelong highly active triathletes and physically inactive controls, but did show an increase in the total surface areas of tibia and patellar cartilage among the athletes[37]. Because the area and thereby the volume expand, exercise could preserve the optimal (normal) thickness and thereby deformation capacity as well, and because of the larger surface area, the AC is able to withstand a greater amount of loading and stress[6]. Using MRI, 12 weeks of either endurance or strength exercise in women aged 40–55 years showed no change in thickness or area in knee cartilage[38], while 10 weeks of running showed improvement in composition using delayed gadolinium-enhanced MRI of cartilage index in knee cartilage in women aged 20–40 years[39].

The deformation capacity in groups of different training status, i.e., professional weight lifters, bobsled sprinters, and untrained controls, was not shown to differ[40]. However, increased thickness of the patellar cartilage but not in the remainder of the knee was found with the surface area being equal to controls[41]. The cartilage of the patella shows a "dose-dependent" deformation with increased loading and range of motion, whereas the tibial and especially the femoral cartilage do not[40]. Eighty women aged 50–66 years with mild knee OA have, in 2 randomized controlled trials, been subjected to longterm (1 yr) high-impact exercise, which

showed improvement of patellar cartilage[42] without any change in femoral or tibial cartilage[43]. The high-impact exercise was measured using an accelerometer, showing little of the impact in the very high region, thus staying within "the safe loading zone" comparable with jumps and sprints[43].

Thus, it seems that a trend exists toward increased patellar cartilage thickness in athletes performing power sports, and another trend exists toward larger tibial and patellar surface areas from endurance sports. Further, it appears that younger individuals are able to gain an effect of exercise quicker than older individuals, though the aforementioned results used different MRI techniques and exercise protocols.

Moderate mechanical loading and cell signaling. PCM and the chondrocyte constitutes a metabolically active unit called a chondron with the presence of collagen type VI and the PG perlecan, which all form a mesh-like capsular ultrastructure that modifies the mechanical load reaching each chondrocyte regardless of location[44]. Fibroblast growth factor 2 (FGF-2) is found in perlecan's large content of heparan sulfate, and when loaded, FGF-2 is brought into chondrocyte vicinity thereby activating the FGF receptor, which through ERK5 produces an anabolic response (Figure 2)[45].

Mechanical stimulation of chondrocytes from human cultures releases IL-4 because of activation of stretch-activated ion channels and integrins and allows IL-4 to work in an auto- and paracrine manner[46]. Integrins are transmembrane proteins capable of activating internal cell signaling and connecting the chondrocyte to the ECM by forming bonds to, for example, collagen type II, VI, and fibronectin[47]. IL-4 leads to increased expression of aggrecan and decreased MMP-3 through the activation of phospholipase C and protein kinase C (PKC; Figure 2)[48]. IL-4 has an antiinflammatory effect and suppresses both IL-1 and tumor necrosis factor-α (TNF-α) by enhancing the breakdown of mRNA for NF-κB[49].

IL-10 also has antiinflammatory effects reducing both the formation of IL-1 and TNF-α by suppressing the translocation of NF-κB, and thus IL-4 and IL-10 work additively[49]. Enhanced expression of IL-10 has been found following a single instance of resistance exercise in patients with OA, and this response has been proposed as a contributory factor for the beneficial effect of exercise on OA[50]. Finally, in rats, moderate loading shows increased lubricin regardless of age[51], along with reduced apoptosis[52].

Overloading and the effect on cartilage composition. Bovine explants containing AC and subchondral bone exposed to mechanical load *ex vivo* have shown that destruction arises in stages depending on intensity: The earliest damage is softening without collagen loss, next follows collagen loss without visible damage, and finally macroscopic destruction appears[31]. The physiological interval ranges from 1–3 MPa to 5–7 MPa on average, corresponding to mild to moderate load, which leads neither to softening nor damage. Softening occurs in the range of 9–15 MPa, resulting from the loss of fibrillar cross links, while nonvisual collagen damage follows at 11–36 MPa[31], characterized by the loss of PG with the loss of collagen organization and crosslinks from SZ leading to swelling due to an increased amount of water[36].

In dogs, overloading because of running showed diminished GAG content both in SZ and IZ, along with softening because of the change in the organization of the collagen network and subchondral bone remodeling[36]. This results in DZ calcification by tidemark duplication in superficial direction. Finally, the collagen network itself is exposed and broken down, thus marking the irreversible transition to OA[3].

A model predicting occurrence and progression of knee OA shows that collagen network damage initiates OA because of abnormal joint mechanics and excessive loading, and progresses depending on body weight[53]. Of interest, during gait, the load in normal-weight individuals never exceeded 7 MPa, while in overweight people it was always between 7 and 15 MPa. The model did not adjust for age or specific physical activity, which would influence the susceptibility and the actual mechanical load in the knee.

Excessive mechanical loading could begin as low as 7–9 MPa[31,33,53]. A finite distinction between reversible and irreversible damage is hard to make because inflammation and biomechanical stiffness due to age and genetics also influence AC quality, and thus susceptibility for irreversible damage.

Mechanical overloading and cell signaling. The release of matrix components can be a result of either overloading because of a structural lesion, reduced loading as previously described, or even a product of normal turnover by breakdown enzymes. Fibronectin fragments (FN-f) indicate a damaged ECM, in which the repair process begins with the removal of broken structures before laying down of new ones[47]. FN-f can activate both integrin and Toll-like receptors (TLR) 2 and 4. The binding of integrin receptor α5β1 leads to activity of PKCδ and subsequently to all 3 MAPK, i.e., ERK1/2, c-Jun N-terminal kinase, and p38α, ultimately yielding NF-κB and resulting catabolism (Figure 2)[47]. The binding of matrix-breakdown products such as FN-f to TLR2 and TLR4 leads to myeloid differentiation factor 88–dependent activation of the same 3 MAPK, followed by increased NF-κB and the resulting MMP[54] and inflammation (Figure 2), which further upregulates TLR[55]. Matrix-breakdown products from fibromodulin and decorin (members of the small leucine-rich proteoglycans), collagen, or cartilage oligomeric protein can activate both integrins as well as TLR and the complement system, resulting in the same catabolic effect[3]. On the other hand, TLR2 and TLR4 are inhibited by lubricin[56]. Finally, high-impact loading (10 MPa) is shown to provide an increase in MMP-1 mediated by p38α[33].

As shown simplified and schematically in Figure 2, all catabolic pathways lead to an increased activity of NF-κB, inducing the process of early OA through the effect of IL-1, TNF-α, IL-6, and IL-8, where the chondrocytes acquire a

hypertrophic phenotype that resembles endochondral ossification[57]. It thereby seems that NF-κB could be related to a hypertrophic phenotype, but substantial evidence toward or against this is lacking. It cannot be ruled out that this histological feature could result from increased PG and thus water content in the PCM, giving the impression that the chondrocytes are indeed hypertrophic. If so, the hypertrophic chondrocytes could exhibit mechanical loading different from a normal healthy cell, which would change cell signaling[58]. In any case, a potential coupling between NF-κB and chondrocyte hypertrophy is yet to be investigated. Further, the chondrocytes have an increased anabolic response with synthesis of matrix elements, but also an increased catabolic response with synthesis of degrading enzymes[14] — this might be an attempt at repair, but inevitably results in a reduced matrix.

It is possible that chondrocyte apoptosis occurs as a result of mechanical overloading of the tissue. The proinflammatory mediators cyclooxygenase 2 and inducible nitric oxide (NO) synthase stimulated, for example, by mechanical loading, could lead to increased amounts of prostaglandin E2 and NO, respectively, and thereby to amplified oxidative stress and apoptosis[57]. Further, mechanical overloading of human cell cultures resulted in increased oxidative stress[10], which induces damage to the mitochondria leading to either apoptosis or senescence, thus reducing the number of functional chondrocytes[11].

Collagen type X is usually not present in AC, but is seen in OA along with vascular endothelial growth factor (VEGF). VEGF causes the growth of blood vessels from the subchondral bone and thereby calcification of the ECM, and because of the growth factors (bone morphogenetic protein 2 and transforming growth factor-β), both osteophyte formation[8,57] and bone resorption are seen (Figure 2)[57]. With the destruction of SZ, damage propagates through IZ and DZ, ultimately leaving behind a thin lining of AC providing the radiological characteristics of OA with joint space narrowing, osteophytes, and subchondral sclerosis[59], marking the endstage and final demise of AC.

DISCUSSION

Increasing age leads to a decline in chondrocyte function due to senescence and hence to a diminished capacity for remodeling and maintenance. This lack of tissue turnover and renewal causes accumulation of AGE, leading to increased stiffness because of these fibrillar crosslinks, followed by a decline in AC deformation capacity and quality, and thereby ultimately to an increased susceptibility for destruction. Age is thus a major predisposing factor for the development of OA.

Within a certain degree, AC can adapt to the amount of mechanical influence in the same way as the rest of the musculoskeletal system — moderate use leads to hypertrophy and maintenance of AC quality while immobilization causes atrophy, both primarily because of changes in the content of PG. Many of the underlying cell signaling mechanisms and pathways have been characterized, showing OA to be both a degenerative and inflammatory disease. Somewhat in contrast with other parts of the musculoskeletal system, mechanical overloading of cartilage causes substantial damage to the collagen network and because of a lack of regenerative capacity, this leads to irreversible destruction and is thus the most apparent triggering cause of OA. Because aging is inevitable, moderate mechanical loading is the best tool to maintain cartilage integrity and health, which can be achieved by different types of activities[60], thus reducing or even preventing OA.

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