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Consequences of metabolic and oxidative modifications of cartilage tissue

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Abstract

A hallmark of chronic metabolic diseases, such as diabetes and metabolic syndrome, and oxidative stress, as occurs in chronic inflammatory and degenerative conditions, is the presence of extensive protein post-translational modifications, including glycation, glycoxidation, carbonylation and nitrosylation. These modifications have been detected on structural cartilage proteins in joints and intervertebral discs, where they are known to affect protein folding, induce protein aggregation and, ultimately, generate microanatomical changes in the proteoglycan–collagen network that surrounds chondrocytes. Many of these modifications have also been shown to promote oxidative cleavage as well as enzymatically-mediated matrix degradation. Overall, a general picture starts to emerge indicating that biochemical changes in proteins constitute an early event that compromises the anatomical organization and viscoelasticity of cartilage, thereby affecting its ability to sustain pressure and, ultimately, impeding its overall bio-performance.

Introduction

The health of the joints of the peripheral and axial skeleton, including the intervertebral discs, depends on the integrity of cartilaginous tissues,[1,2] which, in turn, is influenced by the ability of chondrocytes to maintain the extracellular matrix (ECM) of cartilage. Numerous investigations have aimed to identify mechanisms by which ageing and mechanically-driven stresses lead to fissuring, fibrillation and wear of hyaline cartilage and fibrocartilage (Box 1); however, a newer line of enquiry focuses on the roles of metabolically-driven processes

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in cartilage degeneration. Conditions of prolonged oxidative stress (which occur, for example, during ageing and chronic inflammation) and metabolic stress (through diabetes and metabolic syndrome, for example) can induce biochemical changes, including glycation, carbonylation, lipoxidation and nitrosylation, in cartilage structural proteins. These post-translational modifications induce aggregation and/or unfolding of cartilage matrix proteins, which increases their susceptibility to enzymatic cleavage and degradation.[3,4] Loss of these matrix proteins impairs the ability of cartilage to withstand mechanical stresses and therefore renders it even more susceptible to breakdown.[5–8] The resulting loss of cartilage, observed as joint-space narrowing by radiography, in association with corresponding remodelling of subchondral bone, osteophyte formation and variable levels of joint pain, compromises articular performance and represents the common clinical picture of osteoarthritis (OA), the most common joint disease worldwide.

In this Review, we summarize the current knowledge of the biochemical changes in the articular cartilage matrix—focusing on cartilage matrix proteins—that are associated with diabetes, metabolic syndrome and chronic oxidative stress. Although we acknowledge that both oxidative stress and metabolic diseases can directly affect chondrocytes and, in turn, matrix biosynthesis, an in-depth discussion of the effects of these stresses on chondrocytes is beyond the scope of this article.

Articular cartilage

Articular cartilage provides two essential functions for the joint. In the healthy state, this tissue ensures an extremely low coefficient of friction during joint motion, as well as controlling joint alignment and regulating the distribution of mechanical forces across the joint. The mechanical properties required for this function are critically dependent on the molecular architecture of this tissue.

The architecture of cartilage

Although seemingly structurally simple when viewed by microscopy at low levels of magnification, articular cartilage is composed of a complex ECM that is highly organized to serve its biological purposes.[9] On the basis of studies of cartilage samples taken from different species and different joints, four distinct zones can be distinguished, each with its own characteristic matrix appearance (Figure 1).[5,10] The top layer, the superficial zone, known as the gliding surface, has been shown by transmission electron microscopy and scanning electron microscopy to consist of a layer of amorphous material containing lipids, proteins and proteoglycans, which covers a layer of woven type I and type II collagen fibres oriented in parallel to the surface. The gliding surface confers lubrication and resistance to sheer stress. The second layer, the transitional zone, contains flattened chondrocytes and bundles of type II collagen fibres that are oriented tangentially to the cartilage surface. This zone provides an enclosure for proteoglycan aggregates (formed mainly of aggrecan), which bind water molecules and provide resistance to compression. The radial (deep) zone, forming the third layer, comprises type II collagen bundles arranged radially, as well as chondrocytes and aggrecan. Here, the fibres tend to arborize as they extend outward from the bone surface. This zone anchors the cartilage into the fourth zone, the calcified layer that is immediately beneath the tidemark and above the subchondral bone.

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The molecular make-up of articular cartilage

In general, about 70% of the wet weight of cartilage comprises water and about 30% results from the presence of collagens and proteoglycans, upon which the mechanical properties of the tissue are dependent; a large number of other molecules also have important regulatory and organizational roles.

Collagens—Collagens provide the tissue with tensile strength and are arranged in specific configurations to maximize this property. The principal collagen constituent of articular cartilage is type II, although smaller amounts of types III, IX and XI are also found.[11–13] Several other types of collagen are also present at trace amounts at specific times and in selective locations within articular cartilage.

Proteoglycans—Aggrecan, an enormous molecule of $1–3 \times 10^6$ kDa, is the principal proteoglycan component of the ECM. The aggrecan core protein is a 225-kDa structure with three globular domains (G1, G2 and G3) enclosing two extended regions that provide recognition sequences for modification by approximately 30 keratan sulfate chains and 100 chondroitin sulfate chains.[14–16] G1 at the amino (N) terminus mediates binding of the aggrecan molecule to hyaluronic acid, an interaction that is stabilized by link protein.[14] G2, which is highly conserved, does not interact with hyaluronic acid, and might have some role in aggrecan secretion. G3 has been proposed to interact with tenascins and fibulins to organize proteoglycans within the ECM.[17] Aggrecan is important for its fixed negative charge density, which establishes repulsive forces among the chondroitin sulfate and keratin sulfate side chains and results in a high osmotic pressure within the matrix, consequently drawing water into the tissue. The resultant swelling is restrained by the collagen network.[18]

Other molecules—Additional molecules, such as link protein, decorin, biglycan, matrilins, cartilage oligomeric matrix protein (COMP), tenascins, fibulins and many more, are found throughout the cartilage matrix and are known to carry out critical roles in the formation and organization of, as well as intermolecular interactions within, this tissue. It is, therefore, easy to appreciate how impeding the function of these molecules can adversely affect the mechanical properties of healthy cartilage.

The cellular component of cartilage

Cartilage homeostasis is dependent on the biological activities of the embedded chondrocytes, the only cellular component of cartilage. These cells are dependent upon membrane transporters for nutrients and a series of molecules that enable survival in the face of low oxygen tension.[19] The metabolic activities of chondrocytes are responsive to mechanical load,[20] growth factors,[21] and inflammatory cytokines.[22]

Chondrocytes are dispersed widely throughout the avascular matrix; the properties of this matrix vary according to the distance from the embedded chondrocytes. The matrix that immediately surrounds individual chondrocytes—the pericellular domain—contains specialized molecules such as collagen VI, perlecan and little, if any, type II collagen. It is now understood that the pericellular domain functions to connect the metabolic activity of chondrocytes with the mechanical environment of the joint.[11] The matrix further away from

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the chondrocytes, referred to as the interterritorial domain, contains collagen type II fibrils that are interacting with collagens IX and XI. It is this part of the matrix that confers tensile strength on the articular cartilage.[5]

Protein oxidative modification

Post-translational modifications of collagen and aggrecan in articular cartilage are especially important because these molecules have low rates of turnover, so the functional effects of protein modifications on the molecular conformation, stability and function of cartilage persist for a long time. Studies of cartilage in its steady state indicate that the half-life of type II collagen is in the range of 100 years.[23] Turnover of the aggrecan monomer seems to be more rapid, with a half-life of about 3.5 years.[24] However, the aggrecan G1 domain, which binds to hyaluronic acid, seems to turn over much more slowly, with a half-life of about 25 years. It follows, then, that aggrecan turnover generates proportionately more G1 domain fragments and, that, because these fragments occupy binding sites on hyaluronic acid, aggrecan structures of lower quality are formed with ageing. In conditions of stress, turnover rates seem to accelerate as much as 10-fold.[25] Turnover rates also vary in different regions of articular cartilage and are responsive to mechanical load,[26] growth factors,[27] and inflammatory and degenerative conditions, and seem to be more rapid in the chondrocyte territorial domain.[28] Glycation, glycoxidation and lipoxidation reactions are especially important in metabolic disorders, such as diabetes and metabolic syndrome,[29] whereas carbonylation and nitrosylation are often observed in chronic inflammatory conditions.[3]

Stress-associated protein modifications

Oxidation and reduction, or the redox reaction, involves the transfer of an electron from one molecule (the reducing agent) to another (the oxidizing agent). Protein oxidation involves the covalent modification of the primary sequence of a protein directly by reactive oxygen species (ROS) or reactive nitrogen species (RNS), or indirectly by secondary by-products of oxidative stress (Figure 2).[30] Post-translational oxidative modifications can be classified as reversible or irreversible, and biologically can be beneficial, promoting cell survival and tissue regeneration, or detrimental, causing tissue degeneration and cell death.[31,32] Beneficial oxidative modifications are most often associated with reversible alterations in protein structure, and occur during relatively brief periods of oxidative stress, whereas detrimental alterations typically arise in response to sustained oxidative conditions.

Carbonylation—Protein carbonylation is the most common oxidative reaction. It involves the conversion of N-terminal amino acids or amino-acid side chains into aldehyde or ketone groups by the direct action of either ROS or RNS.[33] Protein carbonyl derivatives can also be formed indirectly by reactive carbonyl compounds created by glycoxidation of carbohydrates, lipoxidation of lipids, and by advanced glycation/lipoxidation end-products. These modifications are mostly irreversible and are widely used biomarkers of oxidative stress in ageing and have been assessed in several chronic and degenerative conditions as contributors to tissue degeneration.[34–36] However, the results of studies carried out over the past 10 years indicate that, in certain conditions, carbonylation can have a beneficial role in signal transduction or can offer protection against reperfusion-induced injuries.[32,37]

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Although all amino acids are susceptible to oxidative alterations, arginine, histidine, lysine, phenylalanine, tyrosine, proline, threonine, tryptophan, methionine and cysteine are more prone to undergo oxidation of their side chains than are other amino acids.[36] Cysteine and methionine side chains contain a reactive sulfur group that is a primary target for reversible or irreversible ROS and RNS modifications. Reversible redox modifications of the thiol group of cysteine include S-sulfenation, S-nitrosylation, and S-glutathionylation, as well as the formation of disulfides.[38,39] These reversible cysteine modifications not only have a beneficial role in protecting target proteins from ROS and RNS oxidative damage, they also influence enzyme activity as well as being important in cellular biological functions, such as cell growth and proliferation. By contrast, the formation of sulfonic and sulfenic acids during chronic oxidative stress constitute irreversible modifications that are associated with tissue damage and loss of protein function.[40]

The oxidation of methionine to methionine sulfoxide does not appear to have biological consequences for protein functionality and can be reversed by the action of a cytosolic reductase. Consequently, methionine residues on protein surfaces have been considered to function as ROS scavengers that protect proteins from irreversible oxidative injury.[41] However, the formation of methionine sulfone, albeit a reversible modification through the activity of methionine reductase, has been shown to decrease the activity of plasma protease inhibitors.

Histidine residues are converted to 2-oxohistidine during prolonged oxidative stress, and this modification has been observed for several catalytic enzymes. The indole ring of tryptophan can be readily oxidized by ROS to form kynurenine and formylkynurenine, and by RNS to form nitrotryptophan. Formylkynurenine and nitrotryptophan have been associated with a decrease in protein function. Although phenylalanine is not as reactive as tyrosine, it can also be oxidized to tyrosine or nitrophenylalanine by ROS and RNS, respectively. In the presence of ROS or RNS, tyrosine forms tyrosinyl radicals, which can dimerize to form dityrosine or couple with hydroxyl radicals to form L-3,4-dihydroxyphenylalanine (L-DOPA) through a non-enzymatic reaction. The interaction of tyrosine with nitrogen dioxide will largely form nitrotyrosine. These tyrosine modifications are often observed during acute and chronic inflammation, when levels of ROS and RNS are elevated. Additionally, these tyrosine modifications are all irreversible and very damaging to protein biological activity. In addition to causing protein unfolding and aggregation, they impair tyrosine phosphorylation, a key mechanism in signal transduction (Figure 2).

The most commonly studied oxidative changes in aliphatic amino acids are lysine to allysine aldehyde (which occurs naturally in collagen and elastine through the action of lysyl oxidase); proline to 2-pyrrolidone; and both proline and arginine to their glutamic semialdehydes. The presence of these amino acids in the oxidized form is indicative of prolonged oxidative stress.

Glycation—Glycation, a non-enzymatic reaction that covalently attaches a reducing sugar to an amino acid, occurs mainly when an aldose group of a monosaccharide or a polysaccharide binds to an amino group within a protein. The process mostly involves lysine and arginine side chains, leading to the formation of a Schiff base (Figure 3).[42] This initial

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reaction is still reversible, and it is strictly dependent on the environmental sugar concentration. Over a period of days, the reactants will undergo chemical rearrangement and form early glycation products, which are known as Amadori products (more than 100 of which have been described). These early glycation steps are still reversible on decreasing the environmental sugar concentration. However, subsequent chemical modifications including oxidation, reduction and hydration can induce protein aggregation and crosslinking. This process, known as the Maillard reaction, takes place over a period of months or years, is irreversible, and leads to the formation of advanced glycation end products (AGEs) including hexitol-lysine, carboxymethyl-lysine, pentosidine, and pyrraline (Figure 3; Box 2).[43,44]

Glycoxidation—Glycoxidation is the reaction that occurs between an amino acid and an oxidized sugar.[45] Glycoxidation leads to the formation of AGEs when glucose, fructose or lipids are oxidized into dicarbonyl derivatives known as α-oxaldehydes (for example, glyoxal, methylglyoxal and 3-deoxyglucosone). These intermediates directly interact with lysine and arginine side chains to form imine linkages that will then be converted to α-amino-ketones through Amadori rearrangements.[46]

AGEs, which are very stable, are particularly damaging in two ways: they directly interact with proteins to alter their structure and function; and they interact with the AGE receptor (RAGE), which leads to proinflammatory effects through the extracellular signal-regulated kinase/mitogen-activated protein kinase (ERK/MAPK) signalling pathway and nuclear factor κB (NFκB) activity.[47]

Additional effects on proteins—Oxidative stress and metabolic stress, in addition to causing protein oxidative modifications, can induce amino-acid side-chain cleavage, oxidative cleavage of the protein backbone, and protein crosslinking as new disulfide bonds form between two oxidized cysteines or Schiff-base formation occurs between carbonyl groups on different amino acids (Figure 4).[40] Cleavage and crosslinking both result in the irreversible loss of the biological activity of the protein.[48–50]

Oxidative stress and cartilage structure

At the clinical level

Several clinical conditions vividly illustrate the impact of oxidative stress on connective tissue such as the articular cartilage matrix. An important example is type 2 diabetes mellitus, which affects more than 200 million people worldwide. Although much attention has been focused on glycation-mediated injury to blood vessels, eyes and kidneys, it is now clear that the metabolic consequences of type 2 diabetes, including hyperglycaemia and hyperlipidaemia, affect every organ in the human body, including articular cartilage. This effect on articular cartilage is highlighted by the increased prevalence of OA among people with diabetes independent of their age and BMI, and by a doubling of the need for total joint arthroplasty among this patient population.[51] Indeed, OA is now viewed as part of the metabolic syndrome, which leads to OA in both weight-bearing and non-weight-bearing joints such as those of the hands and shoulders.[2,51–59] Similarly, a high-fat diet has been shown to accelerate post-traumatic OA progression in both weight-bearing and non-weight-

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bearing joints.[60] Moreover, in a mouse model of obesity-associated OA, exercise was shown to protect against OA progression even in the absence of weight reduction.[61] Improvements in glucose tolerance and expression of proinflammatory cytokines that result from exercise seem to act independently of mechanical stress reduction in ameliorating OA.[62]

Several experimental models provide additional evidence that metabolic abnormalities can damage cartilage.[63–67] Moreover, incubating cartilage tissues with threose, ribose or methylglyoxal has been shown to induce the formation and accumulation of AGEs in collagen and proteoglycan molecules.[68–71] Likewise, chondrocytes cultured in high-glucose conditions generate more ROS, which increases oxidative stress in the local environment.[72] Such *in vitro* data have been confirmed in animal studies, as the same array of post-translational modifications occurs on proteins following intra-articular injection of ribose and threose in models of surgically induced OA.[73] Likewise, conditions associated with prolonged oxidative stress, such as chronic infections and chronic inflammatory processes, are associated with early signs of cartilage degeneration, even when the joints are not the primary targets of the autoimmune or inflammatory process.[74]

At the anatomical level

Several anatomical changes have been associated with oxidative injury to cartilage. The superficial layer of cartilage seems most susceptible to change, probably as a result of direct contact with the synovial fluid and the higher proinflammatory activity of the chondrocytes present in this layer.[75] The macroanatomy of cartilage tissue during prolonged metabolic stress and oxidative stress exhibits early changes in the gliding zone, with a thinning of the proteoglycan and collagen layers as well as disorganization of collagen fibre orientation.[64,76] These changes are followed by more extensive degenerative changes in the deeper transitional and radial zones. Microanatomically, a loss of matrix proteins and chondrocytes is observed in all these zones.[64] For example, in streptozotocin-induced diabetes, a statistically significant decrease in the levels of type II collagen and proteoglycans and a concomitant increase in collagen XI levels were observed in articular cartilage.[64] Similarly, the nucleus pulposus from diabetic rats has been reported to show thinning and increased expression of matrix metalloproteinases (MMPs) and RAGE.[77] Additionally, oxidative stress has been reported to depolymerize glycosaminoglycans and to be associated with the degradation of hyaluronan.[78] The sustained release of RNS during chronic inflammatory processes has also been shown to decrease the synthesis of proteoglycans and collagen in cartilage cultures and of glycosaminoglycan in an *in vivo* arthritis model.[79,80] The decrease in the levels of these cartilage ECM components can be attributed to a decrease in chondrocyte synthetic activity as well as ROS-induced and RNS-induced protein oxidative cleavage and the increased activity of MMPs. Additionally, glycation or carbonylation of collagens and proteoglycans induces protein crosslinking, which changes protein secondary and tertiary structure, alters the spatial orientation of fibres and bundles and changes the surface charge and hydrophilic tension of proteins. Overall, the combined experimental data support the notion that, in conditions of increased oxidative and metabolic stress, cartilage undergoes important changes in its composition and molecular organization, including changes in the proportion of the different collagens and alterations in the ratio of collagen to proteoglycan, disruption of spatial orientation and increased protein

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crosslinking, which all alter its functional capabilities. These microanatomical alterations have profound effects that disrupt the carefully designed molecular arrangement of articular cartilage.[64]

Imaging techniques have been used to assess early changes in cartilage tissue. New MRI techniques, such as T1-rho (T1ρ) and T2 MRI, assess the interaction of water molecules with glycosaminoglycans in articular cartilage and indirectly provide information regarding the integrity of the collagen and aggrecan structure of cartilage. Both T1ρ and T2 MRI have been shown to be predictors for progressive cartilage loss in OA.[81,82] The results from a 2013 study indicate that diabetes and increased abdominal girth are associated with higher T2 values (which correspond to an increased loss of collagen integrity).[83] These results show progress towards the development of imaging techniques that could detect very early biochemical damage to cartilage tissues at a time when many of the protein post-translational modifications are still reversible. Thus, early detection could aid therapeutic interventions that are aimed at reversing early glycation, glycoxidation and carbonylation in otherwise healthy cartilage.

At the biochemical level

On a biochemical level, lysine to allysine aldehyde, proline to 2-pyrrolidone, and proline and arginine to their glutamic semialdehyde are the most commonly reported modifications. Pentosidine and 5,6-dioxoglucosone are also often observed on lysine, arginine and histidine side chains in collagen. Glucosepane, a derivative of 6-glucose that is carbon-crosslinked to lysine and arginine, is one of the main AGEs observed in cartilage proteins from patients with diabetes or metabolic syndrome. Over time, such modifications induce collagen and proteoglycan crosslinking as well as oxidative cleavage, both of which change the biomechanical properties of the tissue, causing stiffening of the collagen network.

Oxidative stress and cartilage function

Several studies have assessed the impact of metabolic stress on the mechanical properties of articular cartilage (Box 3). For example, cartilage from the ankle joints of individuals with diabetes was reported to be softer and more permeable than cartilage from those without diabetes.[84] This observation contrasts with an increased stiffness in cartilage resulting from increased collagen crosslinking observed when AGEs are induced *in vitro*.[85] However, the AGEs are likely to act over a very short period of time *in vitro* to mainly cross-link proteins, whereas the broader effects observed *in vivo* are probably mediated through a wider range of matrix protein modifications as well as altered to mechanosignals.[86] Indeed, prolonged oxidative stress reduces the synthesis of proteoglycan and collagen through effects that involve modulation of phosphatase and tensin homologue deleted on chromosome 10 (PTEN). PTEN functions as a negative regulator of phosphoinositol-3-kinase (PI3K)–AKT and ERK/MAPK signalling pathways, which instruct chondrocytes to upregulate matrix protein synthesis.[87,88] PTEN has been shown to be upregulated in chondrocytes prepared from cartilage affected by OA.[88] Additionally, the interaction of AGEs with RAGE in joint tissues is expected to enhance the local expression of inflammatory molecules, such as IL-1α/β and TNF, which are known to inhibit chondrocyte function. It is not surprising, therefore, that metabolic stresses mediated through oxidative pathways would alter the ECM

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of articular cartilage in ways that compromise the ability of this tissue to effectively withstand mechanical forces involved with locomotion and, together, predispose to cartilage degeneration. In this regard, it is noteworthy that joints with such compromised cartilage are more prone to the development of OA when coupled with other causes of this disease. For example, inducing AGEs in the dog knee by directly injecting ribose was found to increase the severity of subsequent OA following transection of the anterior cruciate ligament.[89] Similarly, tears of the anterior cruciate ligament lead to more severe OA in older humans than in younger individuals.[90]

Conclusions

In the past few years, improved proteomic approaches have enabled the analysis of cartilage protein post-translational modifications that are associated with conditions of chronic metabolic and oxidative stress. A picture emerges of a multitude of protein post-translational modifications that remodel cartilage. Although at present the evidence linking glycation, glycoxidation and carbonylation to impaired cartilage performance is largely correlative, it should be noted that the treatment of animal models of diabetes using agents that inhibit the formation or action of AGEs (aminoguanidine, pyridoxamine, LR-90, metformin, ARB inhibitors and hydralazine) has been shown to decrease diabetic complications,[91] including degenerative changes in cartilage.[92] A future goal would be the comprehensive mapping of each of these modifications to generate a cartilage ‘molecular signature’ for each pathological condition and to understand the effects of each signature on the primary, secondary and tertiary structures of cartilage matrix proteins, with the ultimate aim of gaining a better understanding of the relationship between biophysical alterations in proteins and the corresponding changes in tissue microanatomy and functionality.

In terms of the relationship between OA and the accumulation of oxidative modifications in cartilage, several studies indicate that this relationship is more complex than previously thought. Indeed, although all of these oxidative modifications promote cartilage degeneration through their effects on cartilage ECM proteins, a second set of signals is required to induce inflammation and bona fide degenerative OA. These signals could be provided by any of several means. First, AGEs could bind to RAGE present on tissue-resident macrophages, chondrocytes and osteoclasts to activate an inflammatory programme through NFκB and ERK/MAPK signalling.[93,94] Second, ROS could activate NFκB signalling, leading to the production of proinflammatory cytokines, such as IL-1α/β and TNF. Third, ROS and AGEs might upregulate the expression of MMPs, collagenases and aggrecanases, which enhance the activity of catabolic pathways leading to matrix degradation. And, finally, the synthetic activity of chondrocytes could be decreased by oxidative or metabolic stress.[95,96] Thus, the metabolic and oxidative imbalances observed in several chronic diseases initially cause the post-translational modification of cartilage proteins, which is followed by a second event that generates low-grade inflammation; together, both events promote degenerative changes, leading to the OA phenotype.[97]

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Key points

- Diabetes, metabolic syndrome and chronic infections increase glycaemia, lipidaemia and cellular levels of reactive oxygen species (ROS) and reactive nitrogen species (RNS)
- Increased glycaemia, lipidaemia, ROS and RNS induce glycation, glycoxidation, carbonylation and nitrosylation of cartilage proteins
- Biochemical changes in proteins compromise the anatomical organization of cartilage
- Changes in the anatomical organization of cartilage compromise tissue visco-elasticity and, ultimately, the ability of cartilage to sustain pressure and its overall performance
- Protein biochemical changes are an early event in cartilage degeneration

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Box 1

Cartilage

There are three types of cartilage: hyaline cartilage, fibrocartilage and elastic cartilage. Hyaline cartilage is characterized by an abundant glassy matrix and is found covering bone surfaces in synovial joints, within tracheal rings, and as part of the larynx and nose; fibrocartilage, which is found in menisci, the labra of the shoulder and hip, the annulus fibrosus of intervertebral discs and in the pubic symphysis, is typified by abundant collagen bundles; and elastic cartilage, which is found in the external ear, the eustachian tube and the larynx, appears as dense networks of elastin fibres. Hyaline cartilage and fibrocartilage are exquisitely designed for the purpose of distributing mechanical forces across articulating surfaces, absorbing shock and minimizing friction during joint motion.[10,25,98] In this respect, articular cartilage is able to remodel itself to achieve a best-fit articulation that optimally distributes mechanical stresses to subchondral bone.[99]

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Box 2

Post-translational cartilage protein modifications

- Allysine
- 2-pyrrolidone
- Carboxymethyllysine
- Pentosidine
- Deoxyglucosone
- Glucosepane
- Furosine
- Cysteic acid
- Arginine oxidation to glutamic semialdehyde

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Box 3

Cartilage and mechanical stress

In response to the application of a mechanical stress, cartilage does not respond immediately as would a metal spring. Rather, its response is determined by the spring-like properties of its molecular components and the rate at which fluid components egress. Thus, when cartilage is placed under a constant load (creep mode), compression occurs over a period of time. Similarly when cartilage is immediately compressed to a specified degree (relaxation mode), the force required to maintain that compression can be determined as a function of time. These mechanical features of articular cartilage form the triphasic theory for the swelling and deformation behaviours of articular cartilage.[100] The large array of negative charges distributed over proteoglycans attracts positively charged ions and water molecules, and the density of the matrix restricts egress of water molecules during compression. In addition, the fixed negative charges on the chondroitin sulfate and keratin sulfate side chains of aggrecan repel each other electrostatically. These electrostatic factors work together to create the stiffness of healthy cartilage.[101] Their importance is illustrated by changes in compressibility of cartilage during bovine development and human ageing.[18] During bovine growth from the fetal calf to adult cow, the proteoglycan content of articular cartilage was found to remain constant while the collagen content increased 2–3-fold, resulting in a doubling of the compressive modulus (stiffness).[102–104] By contrast, in the ageing human joint, the overall proteoglycan concentration decreases, aggrecan sidechains become shortened, and the collagen framework becomes disrupted, facilitating increased permeability and increased water content, with a corresponding decrease in the compressive modulus.[5]

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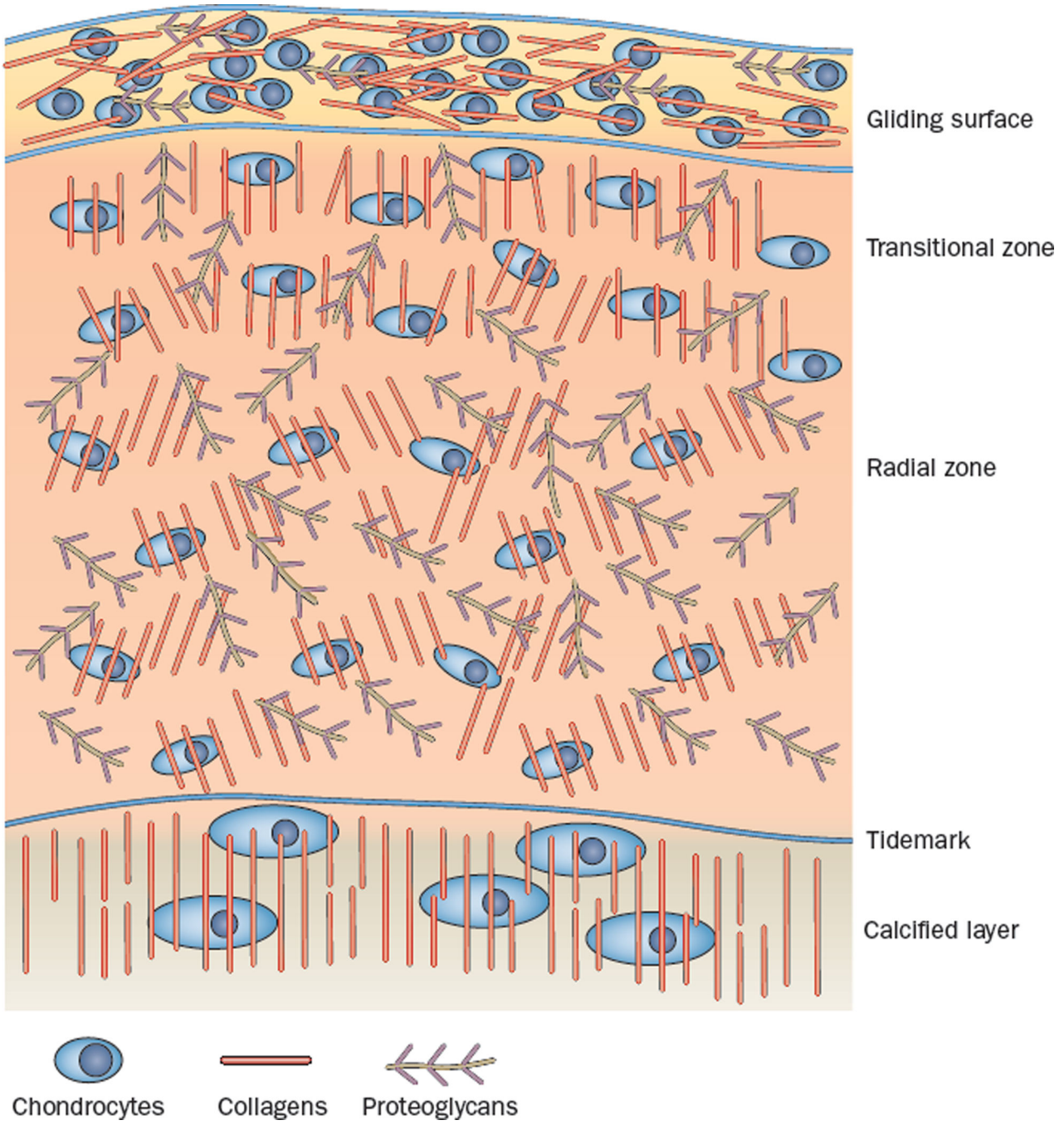

Figure 1.

A schematic representation of articular cartilage. Articular cartilage is organized into four different zones. The surface layer (zone 1), also known as the gliding surface, contains collagen fibres that are organized in parallel to the joint surface, which favours the distribution of joint compressive loads. Zone 1 also contains the highest amount of chondrocytes, which promote matrix synthesis and tissue repair. Zone 2 (transitional) and zone 3 (radial) have a low density of chondrocytes, and collagen fibres are organized obliquely and radially to the joint surface to increase resistance to compressive forces. The

radial zone is the most enriched in proteoglycans. The tidemark distinguishes the radial zone from the calcified cartilage, which anchors the collagen fibrils to the subchondral bone. In the calcified layer, chondrocytes are rare and mostly hypertrophic.

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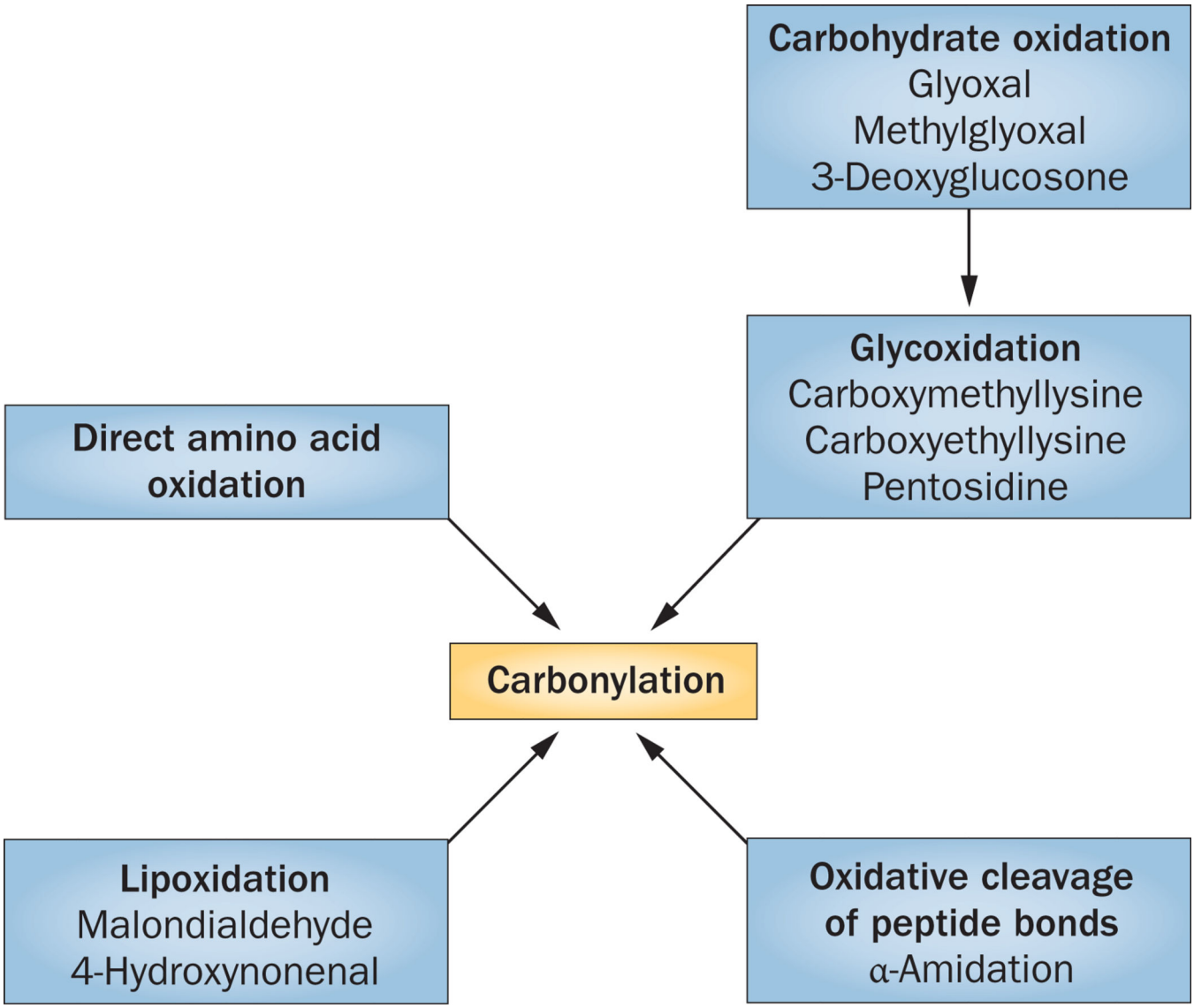

Figure 2.

Overview of the different mechanisms by which proteins can become carbonylated. Carbonyl groups (C=O) can directly modify proteins through direct oxidation of the amino acid side chain or by inducing oxidative cleavage of the peptide bond. Indirect oxidation can occur when carbonyl groups of a previously oxidized lipid (for example, 4-oxo-2-nonenal [ONE], malondialdehyde, acrolein, 2-proponal) or oxidized carbohydrate (glyoxal, methylglyoxal, 3-deoxyglucosone) react with cysteine, histidine and lysine residues, inducing lipoxidation and glycoxidation, respectively.

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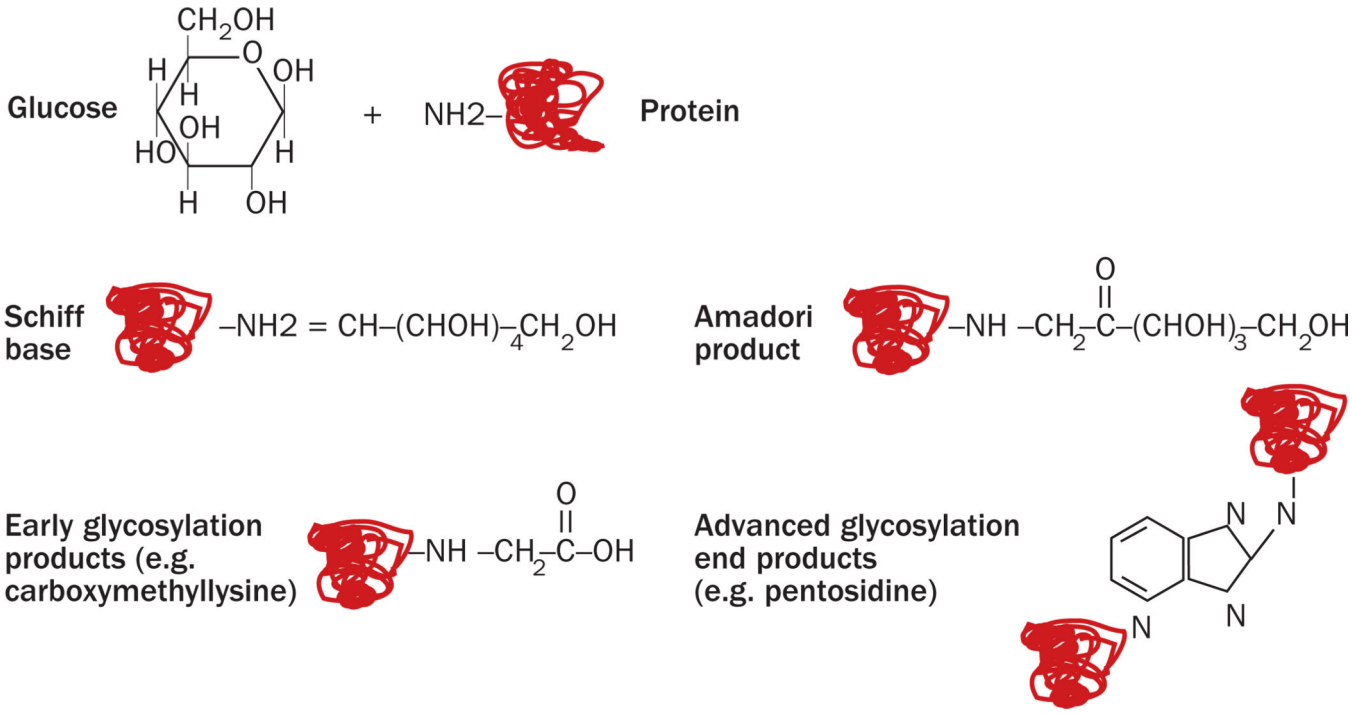

Figure 3.

Step-by-step mechanisms by which advanced glycation end products (AGEs) are formed. In the presence of high levels of circulating monosaccharides and polysaccharides, as occurs in diabetes and metabolic syndrome, the aldehyde group of an aldose (shown here as glucose) can bind to a protein amino group. This non-enzymatic reaction generates a covalent bond (Schiff base). Over time, several chemical rearrangements occur, where the OH group, close to carbon-nitrogen bond binds the nitrogen forming a ketone. Over one hundred rearrangements, known as Amadori products, have been mapped. Subsequent chemical rearrangements, which include reduction, hydration and further oxidation, will generate early and advanced glycation end products.

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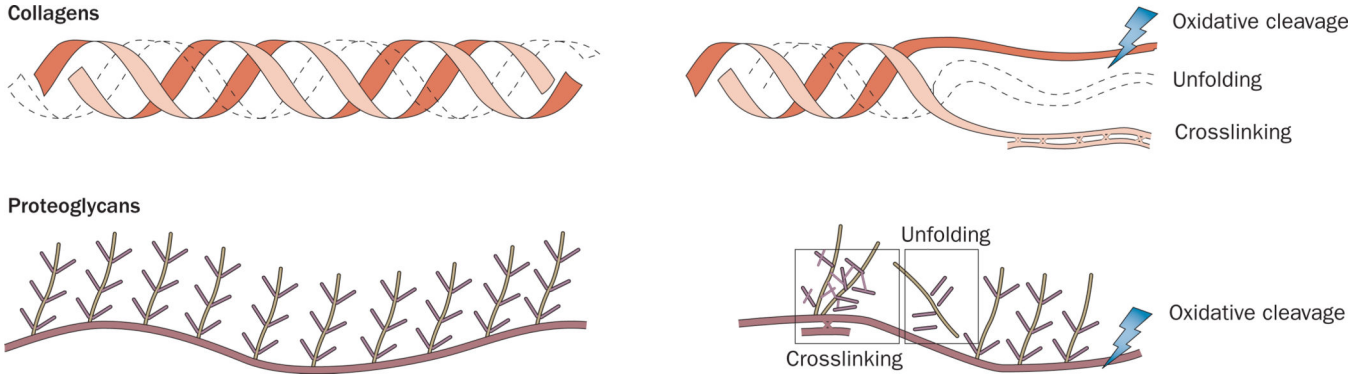

Figure 4.

The effects of oxidative post-translational modifications on the structure of collagens and proteoglycans. Reactive oxygen species and reactive nitrogen species can induce oxidative cleavage by breaking the protein amino-acid bonds or amino-acid side chains. Oxidative post-translational modifications can also induce protein unfolding by steric hindrance or by changing the hydrogen bonds and electrostatic interactions, which keep the proteins correctly folded. Finally, crosslinking between amino acids on the same protein or neighbouring proteins can occur between a free amino group and a carbonyl group.