Diabetes & Metabolic Syndrome: Clinical Research & Reviews 13 (2019) 1877–1883

Contents lists available at ScienceDirect

Diabetes & Metabolic Syndrome: Clinical Research & Reviews

journal homepage: www.elsevier.com/locate/dsx

Review

The role of collagen homeostasis in the pathogenesis of vascular disease associated to insulin resistance

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ARTICLE INFO

Article history:
Received 18 March 2019
Accepted 16 April 2019

1. Introduction

Insulin resistance is associated with devastating vascular damage that is not fully explained by traditional cardiovascular risk factors or hyperglycemia, as the risk of cardiovascular disease remains elevated despite adequate glycemic control [1]. Patients with insulin resistance experience a systemic disorder of collagen homeostasis that may contribute to cause vascular injury among other complications. Collagen abundance, structural properties and fibrillar arrangement are abnormal on the extracellular matrix of every tissue examined, including skin, tendons, bone, skeletal muscle, eye, peripheral nerves, kidney, lung, liver, and blood vessels of any size [2]. Excessive collagen deposition occurs in the interstitial space of tissues and contributes to cause clinical manifestations of diabetes such as fibrotic skin, predisposition to bone fractures, impaired lung function, diastolic dysfunction, and liver disease. Heavy deposits of collagen are present in the capillary basement membrane and the vascular wall of arteries of any size. In capillaries, the ensuing thickening of the capillary basement membrane typifies microangiopathy associated to insulin resistance. In the arterial wall, the striking accumulation of collagen on the tunica media causes intima-media thickening. Histological examinations reveal similar findings (accumulation of disordered collagen) in blood vessel of any size from capillaries to large arteries, suggesting a common pathogenic mechanism to microvascular and macrovascular disease [3,4]. Histological microangiopathy (thickening of the capillary basement membrane) is present in normoglycemic subjects with high risk of developing diabetes but is very rare in hyperglycemic patients with diabetes secondary to pancreatitis. Glucagon deficiency in patients with global pancreas damage prevents insulin resistance from developing and patients with pancreatitis tend to experience exquisite insulin sensitivity. These findings indicate that insulin resistance rather than hyperglycemia is the major factor responsible for vascular injury [5]. Vascular smooth muscle cells synthesize extracellular matrix in the tunica media of the arterial wall. The interaction between smooth muscle cells and the surrounding extracellular matrix is essential to normal structure and function of the blood vessel. Any modification in the composition of the extracellular matrix is sensed by smooth muscle cells via plasma membrane receptors and elicits appropriate cellular adjustment, so that the extracellular matrix regulates cellular function. Collagen is a major component of the extracellular matrix throughout the human body. Alteration of collagen homeostasis in the arterial wall may play a crucial role in the pathogenesis of vascular disease associated to insulin resistance. The cause of the profound disruption of collagen metabolism associated with insulin resistance is unknown [6].

2. Insulin resistance is associated with a systemic alteration of collagen homeostasis

Collagen is a crucial component of the extracellular matrix in human tissues. Three polypeptide chains (named α chains) twisted around each other into a triple helix compose one collagen molecule. A number of distinct α-chains have been identified in humans. Collagen molecules may assemble as homotrimers or heterotrimers of α chains. Mutations in the different genes coding each α-chain lead to a variety of human disorders, including the syndrome of Ehlers-Danlos, characterized by fragility of the blood vessel wall.

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https://doi.org/10.1016/j.dsx.2019.04.019

Amino acids in α chains are arranged in a specific repeating pattern, Gly-X-Y. Every third amino acid in any collagen α chain is glycine. The presence of glycine at this position is essential to bring the three α chains together and form the triple helix. Proline and 4-hydroxyproline occupy frequently the X and Y positions, respectively, and contribute to the stability of the triple helix. Hydroxylation of proline is a key step to collagen formation, as a critical number of prolyl residues must be hydroxylated to form a stable triple helix [7]. Hydroxyproline concentration in a tissue has been used as an index of the amount of collagen present in that tissue. Human collagen contains about 12–14% 4-hydroxyproline while elastin contains about 1.5% [8]. A number of collagen types achieve specific functions in every tissue, but structural and functional differences between them are not well defined. Some types of collagen assemble into fibrils by formation of covalent cross-links between neighboring collagen molecules. Cross-linking of collagen into fibrils occurs in several enzymatic steps. Collagen fibrils may represent the base for hydroxyapatite crystals to deposit physiologically in bone tissue and pathologically in soft tissues such as the arterial wall [7].

Properties of human collagen undergo a marked deterioration with age that may be related with insulin resistance, as decline of insulin sensitivity is a physiological feature of aging. Consistently, age-related collagen modifications mimic those observed in younger patients with insulin resistance. Skin collagen undergoes age-related changes characterized by increased stiffness and fragmentation of collagen fibrils [9]. In human bone, there is an age-related change in collagen content and cross-links from 3 to 89 years of age [10]. Excessive collagen deposition resulting in thickening of the basement membrane of capillaries associated with age has been documented in the retina, the skeletal muscle, and the liver [11–13]. A prominent feature of the aging process in the arterial wall is the progressive accumulation of collagen in the tunica media. In human aortas obtained at autopsy, mechanical measurements suggest that collagen accumulation reduces the elastic response of the arterial wall [8,14,15].

It has been long known that insulin resistance is associated with marked alterations in the abundance, structure, and layout of collagen throughout the human body. Collagen is less soluble by acid and pepsin and less digestible by collagenases and cyanogen bromide in patients with diabetes compared to controls. Defective collagen metabolism in the interstitial space contributes to cause diabetic complications, such as skin changes, restricted joint mobility, tendon diseases, predisposition to bone fractures, and delayed wound healing [3,16]. Alteration of collagen homeostasis precedes the clinical onset of diabetes, having been detected in patients with insulin resistance. In 1975, Hamlin et al. stated: “The possibility must be considered that a connective tissue defect may precede the diabetes and play a role in its pathogenesis.” [17].

In a review of the literature, patients with diabetes have more than three times the odds of tendinopathy compared to controls. Diabetes predisposes to a wide range of tendon diseases, including contracture, rupture, impaired tendon healing, and asymptomatic Achilles tendinopathy [18]. Abnormal collagen structure in tendons may contribute to cause tendinopathy in patients with diabetes. Properties of collagen (evaluated by enzymatic digestion) from the central tendon of the diaphragma obtained at autopsy are altered in patients with diabetes. The collagen fibrils become stiff and insoluble compared to control subjects [17]. The ultrastructure of collagen in extensor tendons (evaluated by X-ray diffraction) shows major changes in patients with diabetes compared to normal tendons [19]. In a cross-sectional study, Achilles tendon stiffness was higher and tendon fibril density was greater in patients with diabetes compared to control subjects [20].

Limited joint mobility occurs commonly among patients with diabetes and may affect any joint, including wrist, elbow, ankle, and spine. Joint stiffness is associated with sclerotic and microvascular complications. Knee osteoarthritis is more frequent in patients with diabetes and the complication rate of total knee arthroplasty is higher compared to controls. Aseptic loosening and need for revision surgery after total knee arthroplasty occur more frequently in patients with diabetes, reducing functional outcome [21].

Patients with diabetes have an increased incidence of intervertebral disc herniation compared to nondiabetic individuals. Collagen is an important component of the intervertebral disc. Type IX collagen accounts for 1–2% of all disc collagen, but its binding to type II fibrils enhances the resistance of the disc. The level of type IX collagen in the intervertebral disc is reduced in patients with diabetes compared to control subjects. This reduction may facilitate the development of disc herniation [22].

A meta-analysis of the literature concluded that the risk of bone fracture is more elevated in patients with diabetes compared to control subjects. Glycated hemoglobin (HbA1c) was not linked to bone mineral density [23]. Bone extracellular matrix is composed of collagen fibrils and mineral. Type I collagen is the most abundant collagen in bone, although types III and V are also present. The elevated risk of bone fracture associated with diabetes is not explained by a reduction in bone mineral density, suggesting that the poor bone quality is related to defective bone collagen [10].

Dura mater is a dense collagen structure. The properties of dura mater collagen obtained at autopsy are markedly altered in patients with diabetes. Compared to normal specimens, dura mater collagen from diabetic patients is much more resistant to solubilization [24].

In a biracial study with Chinese and Caucasian participants, collagen content in adipose tissue (assessed by the level of hydroxyproline) increased with the degree of insulin resistance, evaluated by the homeostasis model assessment of insulin resistance (HOMA-IR) index, in both population groups. The level of hydroxyproline (and the extent of fibrosis) in adipose tissue was higher among more insulin-resistant patients [25]. Analogous results were obtained among obese subjects that underwent hyperinsulinemic euglycemic clamp to evaluate insulin sensitivity. The extent of fibrosis in adipose tissue was higher in more insulin-resistant subjects compared to those more insulin sensitive. Fibrosis of adipose tissue in obese subjects is associated with insulin resistance [26]. In a study that recruited healthy males, the synthesis of type I and type III collagens in the adipose tissue increased in response to insulin resistance. Insulin resistance was induced by overfeeding-related weight gain and assessed by euglycemic hyperinsulinemic clamps. Collagen content was measured in biopsy samples. Insulin resistance was followed by elevated content of type I and type III collagens in the adipose tissue [27].

3. Collagen homeostasis and microangiopathy associated with insulin resistance

Numerous investigations have long documented the presence of systemic capillary involvement in patients with insulin resistance, characterized by collagen accumulation and subsequent thickening of the capillary basement membrane, the histopathological hallmark of diabetic microangiopathy [3,5,28–30]. This alteration precedes the clinical diagnosis of type 2 diabetes, so that capillary basement membrane thickening can be demonstrated in prediabetic subjects prior to detectable hyperglycemia. In contrast, the width of the capillary basement membrane in patients with type 1 diabetes is normal at the time of diagnosis and thickens with increasing duration of the disease. These findings suggest that insulin resistance is a major factor causing microvascular damage [29–31]. Histological examinations reveal that thickening of the

basement membrane occurs in the capillaries of every tissue examined, including retina, kidney, skeletal muscle, skin, liver, lung, and peripheral nerves. Microangiopathy may contribute to cause complications of diabetes such as retinopathy, nephropathy and peripheral neuropathy [3].

3.1. Gingival tissue

In patients with diabetes, basement membrane thickening is identified in terminal arterioles and capillaries from gingival tissue, compared to control subjects. In prediabetic patients, approximately 50% of the terminal arterioles and capillaries show basement membrane thickening. In obese subjects, this alteration is occasionally present while no change is observed in control subjects [31].

3.2. Kidney

Diabetic nephropathy is characterized by thickening of the basement membrane of the glomerular capillaries and expansion of mesangial matrix (Kimmelstiel and Wilson intercapillary sclerosis). Thickening of the basement membrane of kidney tubules and capsular epithelium is also present [32–35]. Mesangial content of type VI collagen has been found 2.8-fold higher in patients with diabetes compared to control subjects [33].

3.3. Eye

Microangiopathy affecting retinal capillaries has been long documented in patients with diabetes. Diabetic retinopathy is characterized by capillary aneurysms and a cluster of exudates and hemorrhages in the retina surrounding the aneurysms. Thickening of the retinal capillary basement membrane is observed [32]. Electron microscopy and histochemical studies performed on human eyes confirm that the retinal capillary wall is thickened in patients with diabetes compared to normal samples [11]. Microvascular abnormalities in the choroid have also been identified in patients with diabetes [36]. The prevalence of brain microangiopathy (evaluated as cerebral microbleeds on magnetic resonance imaging) is higher in diabetic patients with proliferative retinopathy, compared to nondiabetic subjects and diabetic patients without retinopathy, suggesting that retinal changes may indicate a generalized microangiopathy [37].

3.4. Nervous system

Brain small vessel disease expressed on magnetic resonance imaging as lacunar infarctions, white matter lesions and microbleeds is common in patients with diabetes compared with healthy individuals. Cerebral microangiopathy is associated with abnormal skin capillary perfusion (assessed by capillary microscopy), suggesting that cerebral microangiopathy is a manifestation of generalized microvascular dysfunction [38,39].

The capillary basement membrane of sural nerves is thicker (by 52.9%) in patients with diabetes compared to control nerves. Endoneurial microangiopathy is closely associated with clinical diabetic polyneuropathy [40]. Similarly to the capillary basement membrane, the thickness of the perineurial cell basement membrane (assessed by electron microscopy) is greater in sural nerves from patients with diabetes compared to nondiabetic subjects. Thickening of perineurial cell basement membrane may precede the recognition of hyperglycemia. In sciatic nerve samples from patients with diabetes, aggregation of type VI collagen fibrils is identified adjacent to the basement membranes of perineurial cells [41].

3.5. Skin

Microangiopathy targets the skin of patients with diabetes. Histological examinations show thickening of the basement membrane of dermal capillaries and deposition of hyaline periodic acid-Schiff (PAS)-positive material on the normally fibrillar area external to the endothelial cell, compared to normal control biopsies [28,42]. Dermal collagen is also abnormal in the interstitial space. Atomic force microscopy images of normal human skin reveal tightly packed and well-organized dermal collagen fibrils. In contrast, dermal collagen fibrils are fragmented and disorganized in patients with diabetes [9]. Patients with diabetes show increased cross-linking of the skin collagen fibrils. Skin collagen is stiffer and more insoluble than that of nondiabetic individuals [20]. On skin biopsy samples, thickening of the epidermis and dermis with collagen accumulation is observed in patients with diabetes compared to controls. Type III collagen is the predominant type deposited in diabetic skin. Fragmented and cross-linked collagen contributes to cause clinical abnormalities such as thick tight skin typical of diabetes [21]. Fibrocytes from diabetic patients do not increase the expression of type I collagen on stimulation, unlike normal fibrocytes. This abnormality may contribute to explain impaired wound healing [43]. Diabetes is a frequent finding among patients with cutaneous collagenous vasculopathy, an idiopathic skin microangiopathy characterized by dilated dermal capillaries with walls thickened by hyaline material containing collagen IV by immunohistochemistry. Similarly, collagenous colitis has been reported in patients with type 1 diabetes. Endoscopic biopsies showed marked thickening of the subepithelial collagen plate (Masson's trichrome positive, Congo red negative) in samples from stomach, duodenum, terminal ileum, and colon [44].

3.6. Skeletal muscle

Thickening of the capillary basement membrane in the skeletal muscle is a very constant finding among patients with diabetes. The width (measured by electron microscopy) of the capillary basement membrane is over twice compared to normal subjects. Hyperglycemic patients with diabetes due to pancreatitis rarely experience this alteration while thickening of the capillary basement membrane is found in approximately 50% of normoglycemic subjects with family history of diabetes, suggesting that insulin resistance rather than hyperglycemia is the major factor responsible for microvascular disease [5,29]. Follow-up skeletal muscle biopsy examinations on the subjects with family history of diabetes revealed that patients who developed diabetes (47%) during the follow-up period showed progressive thickening of their capillary basement membrane compared to the initial measurement [45]. Collagen synthesis increases in the skeletal muscle of healthy males in response to insulin resistance. Participants underwent overnutrition to 10% weight gain. Skeletal muscle biopsies were obtained to measure collagen content and hyperinsulinemic euglycemic clamps were performed to assess insulin sensitivity. Overfeeding and weight gain worsened insulin sensitivity. In response to insulin resistance, an increase in the mRNA level of type I, III, IV, V, and VI collagens was observed in the skeletal muscle. Type III and type VI collagen protein levels were unchanged [27]. Comparable results were documented in healthy subjects who underwent lipid infusion to induce insulin resistance, compared to saline infusion. Following insulin resistance, an overexpression of type I and type III collagen genes with an increase of mRNA levels was observed on biopsy samples of skeletal muscle [46]. Consistently, the amount of type I and type III collagens in skeletal muscle (measured in biopsy samples) is increased in patients with insulin

resistance (assessed by hyperinsulinemic euglycemic clamp) compared to insulin-sensitive subjects [47].

3.7. Heart

Myocardial microangiopathy is a complication of diabetes. Histological examination of autopsy samples document intense PAS-positive deposits on the smallest branches of coronary arteries in patients with diabetes compared to control subjects. The presence of PAS-positive material in the small vessels of the myocardium was closely related to the presence of nodular glomerulosclerosis in the kidney [48]. In addition to myocardial microangiopathy, interstitial fibrosis with accumulation of type III collagen is typically present in the myocardium of patients with diabetes compared to controls [49,50]. Type I collagen mRNA level is elevated by twofold in cardiac fibroblasts (cells that synthesize extracellular matrix) derived from patients with type 2 diabetes compared to controls [51]. The association between insulin resistance and serum markers of collagen synthesis has been investigated in obese subjects. Serum procollagen type III aminopeptide was measured and insulin sensitivity was evaluated by the HOMA-IR index. Regression analyses showed that serum procollagen type III aminopeptide level was independently correlated with HOMA-IR and HDL-c, suggesting that markers of cardiac fibrosis are related to insulin resistance [50]. Myocardial interstitial fibrosis impairs diastolic function hindering ventricular relaxation and contributes to increase the risk of heart failure in patients with insulin resistance. In addition, interstitial fibrosis may predispose to cardiac arrhythmias. Impaired diastolic function has been reported in patients with insulin resistance and obesity [50].

3.8. Lung

Diabetic microangiopathy occurs in the lung. Electron microscopy examination on human lung samples shows thickening of the alveolar capillary basement membrane. In addition, the thickness of the basement membrane of bronchial epithelial cells is greater in patients with diabetes compared to control subjects and interstitial fibrosis with collagen accumulation is observed. There is no close correlation between fasting blood glucose or HbA1c level and basement membrane thickness in lung tissue [35,52]. Patients with insulin resistance suffer from subclinical lung dysfunction long before the clinical onset of type 2 diabetes. Cross-sectional studies in different population groups reveal an independent association between insulin resistance and impaired ventilatory function, including reduced forced vital capacity (FVC) and forced expiratory volume in 1 s (FEV1). It has been suggested that measurement of lung function by spirometry may be useful to estimate the progression of systemic microvascular disease in patients with insulin resistance [53]. In nondiabetic subjects, the decline of FVC and FEV1 was prospectively investigated during 5 years. After adjustment for confounders, subjects who developed diabetes during the follow-up period had greater decline of ventilatory function compared to those who did not develop diabetes [54]. The prospective association between pulmonary function and incidence of diabetes was investigated by the National Health and Nutrition Examination Survey Epidemiologic Follow-Up Study, a cohort study of US adults. Individuals with restrictive lung disease are at risk for developing diabetes, but obstructive lung disease is not associated with diabetes incidence [55].

3.9. Liver

In the normal liver, the perisinusoidal space of Disse contains occasional small deposits of collagen fibrils including types I, III, IV, and XIX. Patients with insulin resistance typically develop a non-cirrhotic form of hepatic sinusoidal fibrosis (diabetic hepatosclerosis) that is not associated with nonalcoholic steatohepatitis. On histological examination, the most striking feature is the presence of dense perisinusoidal fibrosis. On electron microscopy, an increase in the amount of collagen fibrils within the space of Disse with abundant collagen bundles and segments of perisinusoidal basement membrane-like deposition are observed among patients with diabetes while collagen deposits are absent in control subjects. Perisinusoidal collagen accumulation is associated with hyaline thickening of small hepatic artery branches and perivascular fibrosis composed mainly of fibrillar collagen. Patients with diabetes who develop hepatic sinusoidal fibrosis (collagenization of the space of Disse) usually have evidence of microvascular disease, such as retinopathy, nephropathy, and peripheral and autonomic neuropathy. By immunocytochemistry, an increase in type I and type III collagens is identified that corresponds to the collagen bundles seen under electron microscopy. Type IV collagen shows linear segments of perisinusoidal staining, imparting a basement membrane-like appearance. Stellate cell activation is observed in patients with diabetic hepatosclerosis [56,57]. Hepatic perisinusoidal fibrosis has been observed in obese patients as well. Electron microscopy examination of liver biopsy samples from obese patients shows deposition of abundant collagen fibrils and electron-dense material resembling basement membrane within the space of Disse [57].

4. Collagen homeostasis and macroangiopathy associated with insulin resistance

Patients with insulin resistance develop vascular damage characterized by atherosclerosis and medial calcification (Mönckeberg's sclerosis) that may be explained by defective extracellular matrix homeostasis on the arterial wall. Fatty streaks or intimal xanthomas are focal accumulations of fat-laden macrophages (foam cells) in the arterial intima. Microscopically, the media is preserved and extracellular matrix accumulation has not been found on these lesions. Intimal xanthomas are flat lesions that usually regress. The extent of fatty streaks in young persons does not predict the extent of raised lesions (fibrous plaques) in later life. Potentially progressive atherosclerotic lesions include intima-media thickening of the arterial wall and fibrous plaques. Vascular calcification is associated with either of them. Intima-media thickening is a diffuse widening of the intima-media of the arterial wall. On histological examination, the lesion is characterized by collagen accumulation. Microcalcification foci are a very common finding. There are no foam cells. Fibrous plaques are focally distributed in the same area that the intima-media thickening lesion. Fibrous plaques are raised lesions that protrude into the lumen of the artery. Dense bundles of collagen are found in every fibrous plaque. The densely fibrotic areas are free from large accumulations of fat. Small lacunar spaces are commonly seen in this fibrous structure. Calcification can be seen in the plaque and in the adjacent artery wall. In advanced fibrous plaques, the collagenous extracellular matrix forms a fibrous cap that encloses a necrotic core containing debris including lipids. The fibroatheroma plaque contains varying degrees of infiltration by macrophages and lymphocytes that represent a protective response. Advanced fibrous plaques with a necrotic core (fibrous cap atheroma) may undergo complications such as rupture or thrombosis that cause life-threatening occlusive episodes [58–66]. Intima-media thickening and fibrous plaques are commonly complicated by arterial wall calcification. Vascular calcification is driven by smooth muscle cells in response to altered extracellular matrix and involves deposition of carbonated hydroxyapatite (calcium phosphate) on the extracellular matrix.

Arterial calcification is observed microscopically in the intima-media thickening lesion, indicating that microcalcification foci develop at the beginning of the atherosclerosis process [66–69]. In analyses of human coronary arteries conducted to quantify the calcium-to-phosphate ratio on the tissue, microcalcifications composed of amorphous calcium phosphate can be demonstrated at a preatheroma stage, suggesting that the formation of calcium phosphate granules is an early event in the atherosclerotic process [70]. Electron microscopy examination on human aorta samples obtained at autopsy detects zones of microcalcification on intima-media thickening lesions, confirming that calcified deposits are formed at an early stage of atherosclerosis development [67].

Autopsy studies reveal that atherosclerotic lesions begin to appear in young population groups indicating that preventive actions need to be implemented early in life. Among them, compelling evidence derived from prospective studies has demonstrated that animal protein increases the risk of both type 2 diabetes and cardiovascular disease by causing insulin resistance. Restriction or discontinuation of animal protein consumption is crucial to reduce vascular disease associated with insulin resistance [61,65].

In a comprehensive histopathologic study of medium-to-large caliber blood vessels in 100 autopsy subjects, vascular tissue was analyzed to determine the percent fibrosis of over 700 vascular segments. The percent fibrosis (% collagen) of the tunica media was strongly correlated within subjects across all blood vessels suggesting that fibrosis is a global process independent of the vessel [71].

Investigation on human atherogenesis has been accelerated by the development of models of human atherosclerotic lesions and induced pluripotent stem cell technology that makes available human vascular cells generated from induced pluripotent stem cells [6,72].

4.1. Role of collagen homeostasis in the pathogenesis of macrovascular disease associated to insulin resistance

In 1883, Thoma and Kaefer suggested that medial damage played a primary role in the development of atherosclerosis. Medial lesions resulted in focal areas of injury which became filled up by a fibrotic repair process leading to atherosclerotic plaques. Medial thinning was later confirmed to be an initial typical finding in fibrous plaques [4,58]. Smooth muscle cells in the medial layer synthesize extracellular matrix. Abundant components of human arterial extracellular matrix are elastin, proteoglycans and collagen. Additional constituents include enzymes that degrade extracellular matrix (such as matrix metalloproteinases) and their inhibitors, and molecules that regulate the assembly of collagen into fibrils, such as small leucine-rich repeat proteoglycans including decorin. The extracellular matrix interacts with smooth muscle cells via cell membrane receptors such as integrins. Changes in extracellular matrix composition are detected by smooth muscle cells and elicit pathophysiological responses. Molecular interactions between components of the extracellular matrix (such as collagen) and smooth muscle cells contribute to the normal function and adaptive responses to damaged arterial wall. Dysfunctional overproduction of collagen in response to an unknown injury leads to the structural changes that characterize vascular damage associated to insulin resistance [73,74]. Matrix degrading proteins and their inhibitors or other molecules that regulate collagen assembly into fibrillar structures may play a role modulating arterial damage, but their impact has not been defined [75]. There is a pathological “diabetes phenotype” in vascular smooth muscle cells. Smooth muscle cells isolated from patients with diabetes exhibit abnormal morphology and greater adhesion and migration in cell cultures compared to those from nondiabetic subjects [76].

Collagen composition, distribution, and mechanisms of fibrillar assembly on the normal wall of human arteries are not well known. Type I and type III collagens are the most abundant types in the medial layer. Type IV and type V collagens have been found around individual smooth muscle cells in the medial layer and surrounding endothelial cells in the intima forming a basement membrane. Unlike type IV, type V collagen is also distributed throughout the extracellular matrix in the aortic media [15]. Type II collagen has been detected in small amounts in the media of human aorta sections. The average content of type II collagen is approximately 1% of total arterial collagen while collagens types I, III, and IV amount to 99% [77]. Type XIX collagen has been identified on the endothelial basement membrane of human blood vessels [34].

Patients with insulin resistance show abnormal collagen homeostasis in the arterial wall including elevated collagen content, disordered collagen arrangement, and altered collagen composition, which contributes to cause vascular injury. Intima-media thickening and fibrous plaques are characterized by accumulation of collagen. The measurement of hydroxyproline reveals an increase of the collagen content in fibrous plaques, comprising 40% of the total protein versus 25% in normal human aorta. Collagen constitutes as much as 60% of the total protein in advanced lesions [8,15,78]. Fibrous plaques show disordered and shapeless collagen structures, in contrast to the organized assembly of fibrillar collagen in the normal arterial wall. Collagen fibrils degenerate in the dead tissue zones of atheromatous plaques [15]. Collagen distribution is not uniform within the fibrous plaques. These lesions consist of regions with abundance of type I and type III collagens and areas deficient in these collagen types [15,73,79]. The amount of type IV collagen increases with the advance of atherosclerosis. Heavily thickened type IV collagen structures surrounding individual smooth muscle cells are found in fibrous plaques [15]. The abundance of type II collagen increases markedly in atherosclerotic lesions, particularly in samples with calcification. The amount of type II collagen is higher in the tissue around the calcium deposit [77]. The relationship between collagen and arterial calcification has been investigated *in vitro* in human carotid atherosclerotic plaques and three-dimensional collagen hydrogels (that mimic structural features of the atherosclerotic fibrous cap). There is an inverse relationship between collagen content and the size of microcalcifications in human fibrous plaques. The microcalcifications aggregate within fibrous plaques in gaps between collagen fibers. Similarly, increasing collagen content in the hydrogels reduces microcalcification size by 90% compared to the samples without collagen. Further, collagen degradation promotes microcalcification. Localized collagen decay within the plaque allows extracellular vesicles to accumulate and initiate the calcification process. Then, microcalcifications serve as building blocks for larger calcifications when normal collagen is deficient [80].

5. Summary

Insulin resistance is associated with systemic impairment of collagen homeostasis that affects the extracellular matrix of human tissues including blood vessels of any size. Excessive deposition of abnormal collagen on the interstitial space contributes to cause skin abnormalities, bone fractures, tendon disorders, lung dysfunction, diastolic dysfunction, and liver disease. Accumulation of defective collagen on the capillary wall causes microvascular disease including retinopathy, peripheral neuropathy, and nephropathy. Collagen accumulation on the medial layer of large arteries produces intima-media thickening. Fibrous plaques develop in regions of intima-media thickening with further collagen deposition. Both lesions are associated with calcification of the arterial wall.

Funding

There was no financial support for this work

Conflicts of interest

The authors declare that they have no conflict of interest.

Acknowledgement

We are pleased to acknowledge the continuous support from Ms Gema Souto-Adeva.

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