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Experimental diabetes modulates collagen remodelling of joints in rats

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Summary. The aim of this study was to evaluate extracellular matrix components in articular cartilage, ligaments and synovia in an experimental model of diabetes. Young *Wistar* rats were divided into a streptozotocin-induced (STZ; 35 mg/kg) diabetic group (DG; n=15) and a control group (CG; n=15). Weight, blood glucose and plasma anti-carboxymethyllysine were measured 70 days after STZ infusions. Knee joints, patellar ligaments, and lateral and medial collateral ligaments were isolated and stained with hematoxylin-eosin and Picrosirius. The total collagen content was determined by morphometry. Immunofluorescence was employed to evaluate types I, III, and V collagen in ligaments and synovial tissues and types II and XI collagen in cartilage. Results: Higher blood glucose levels and plasma anti-carboxymethyllysine were observed in DG rats when compared to those in CG rats. The final weight was significantly lower in the DG rats than in the CG rats. Histomorphometric evaluation depicted a small quantity of collagen fibers in ligaments and articular cartilage in DG rats, as well as increased collagen in synovial tissue. There was a decrease in cartilage proteoglycans in DG rats when compared with CG rats. Immunofluorescence staining revealed an increase of collagen III and V in ligaments, collagen XI in cartilage, and collagen I in synovial tissue of DG rats compared with CG rats. Conclusion: The ligaments, cartilage and synovia are highly affected following STZ-induced diabetes in rats, due the remodeling of collagen types in these tissues. This process may promote the degradation of the extracellular matrix, thus compromising joint function. Our data may help to better understand the pathogenesis of joint involvement related to diabetes.

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Introduction

Chronic hyperglycemia results in the development of peripheral neuropathies, gastrointestinal and renal dysfunctions, immunodeficiency, microvascular lesions and wound healing disorders (Le Pape et al., 1981; Brennan, 1989; Stefek et al., 2000; Wang et al., 2003; Chbinou and Frenette, 2004). It is well known that painful or stiff shoulders, Dupuytren's contracture, tendonitis, joint stiffness, trigger finger, carpal tunnel syndrome, and osteoarthritis are prevalent musculoskeletal disorders in diabetes mellitus (DM) patients (Aydeniz et al., 2008; Sarkar et al., 2008; Fujinaka, 2009; Kameyama et al., 2009). The articular structures have in common a large amount of extracellular matrix (ECM), including collagens, in different proportions and types depending on the mechanical stress over the analyzed tissue. Collagen types II, IX, and XI account for about two-thirds of the dry weight of the articular cartilage in adults and provide tensile strength to the cartilage. In addition to collagens, cartilage also contains proteoglycans, of which the predominant type is aggrecan, a large chondroitin sulfate proteoglycan, and water (Linsenmayer, 1991; McGonagle et al., 2010). The synovial tissue, a modified tissue space formed by lining cells and the synovial membrane, contains large quantities of type I collagen and smaller quantities of types III, IV, V, and VI collagen. Finally, the tendons and ligaments are mainly comprised of type I collagen fibers, with small quantities of collagen types III, IV, and V, in a matrix surrounded by connective tissue (Turk et al., 1999; Teodoro et al., 2004; Sarkar et al., 2008; Fujinaka, 2009).

It is well known that collagen is extremely

susceptible to modification by nonenzymatic glycation (Le Pape et al., 1981; Brennan, 1989; Linsenmayer, 1991). This reaction is prevalent under hyperglycemic conditions, such as in patients with DM, chronic renal failure and detoxification failure mediated by inflammation. Further, the hyperglycemic conditions enhance concentration of glycation reaction intermediates. Advanced glycation end products (AGE) can be formed in the late stage of the glycation reaction, inducing crosslinking of collagen and alteration of collagen solubility and proteolytic digestion. These effects are manifested as stiffness in skeletal muscles and joints in humans (Semba et al., 2010).

The receptor for AGE interaction with carboxymethyllysine (CML), which is one of the major AGEs formed in vivo, induces the generation of reactive oxygen species (ROS) and nuclear factor kappa B (NF-κB). Reactive oxygen species (ROS) are highly reactive oxidizing agents. When ROS are overproduced or when the body fails to eliminate ROS in excess, oxidative stress arises, during which ROS accumulate and damage cells and tissues. This results in the transactivation of inflammatory genes, metalloproteases, and apoptosis (Turk et al., 1999; Stefek et al., 2000; Tonra et al., 2001; Bolzán and Bianchi, 2002; Chbinou and Frenette, 2004).

The pathology of diabetes is associated with major effects on connective tissues and has a significant impact on the development and outcome of cartilage, bone, ligament and tendon diseases. An improved understanding of the mechanisms by which diabetes alters connective tissue metabolism may lead to the development of more effective preventive and therapeutic interventions (Burner and Rosenthal, 2009). From this standpoint, a streptozotocin (STZ)-induced model of DM in rats is useful to study diabetes resulting from selective damage to pancreatic cells (Le Pape et al., 1981; Novelli et al., 2001; Tonra et al., 2001).

Although the precise etiology of diabetes-associated musculoskeletal disorders remains uncertain, there is evidence that hyperglycemia may accelerate nonenzymatic glycation and collagen deposition (Turk et al., 1999). We hypothesize that high blood glucose in diabetic rats may lead to histoarchitectural changes in connective tissue around the knee. To address this issue, we investigated the remodeling of cartilage, ligaments and synovial tissue in a STZ-induced diabetes animal model.

Materials and methods

Animals and experimental protocol

This study was approved by the Ethics Committee for the Analysis of Research Projects (CAPPesq) Protocol #1060/06-CEP-UMESP, #211/06. All animals received humane care in compliance with the experimental protocols of the Ethical Principles in Animal Experiments adopted by the Brazilian Association of Animal Testing (COBEA).

Two-month-old male *Wistar* rats weighing 200-250g were randomly assigned into a control (CG, n=15) or a diabetic group (DG, n=15). During the experiments, animals were kept in an animal house with a 12 hour light/dark cycle and controlled temperature (22±2°C). Water and standardized food were provided *ad libitum*.

Blood samples were obtained from the tail veins of all animals, and were assayed for plasma glucose (Accu-Chek® Advantage blood glucose meter; Roche, Indianapolis, Indiana) before STZ administration.

Under intraperitoneal anaesthesia with pentobarbital sodium 60 mg/kg body weight (Hypnol®), diabetes was induced through acute intravenous infusion of STZ (35 mg/kg body weight; Sigma, St. Louis, MO) via the tail vein. STZ was diluted in 0.9% sodium chloride solution (Baxter Hospitalar Ltda, Brazil), adjusted to pH 7.2-7.4, and immediately infused into the tail vein. The control rats were injected with vehicle alone. Rats with a blood glucose level of 300 mg/dl or greater were considered to be diabetic.

The animals were maintained under diabetic conditions for 70 days after infusion with STZ and did not receive insulin during this time (Tonra et al., 2001; Novelli et al., 2001).

The body weight of the rats was monitored weekly and blood samples were obtained from the tail vein 1 and 70 days post-STZ infusion and assayed for blood glucose (Accu-Chek® Advantage blood glucose meter; Roche, Indianapolis, Indiana). Plasma anti-CML antibody production was measured at 70 days post-STZ infusion using an ELISA (Circulex®; Protocol #8068; Cyclex Co., Japan). Animals were fasted for 6 hours prior to each blood sampling, except for the first collection, when animals were fasted for 12 hours prior to blood sampling to measure basal blood glucose.

At the end of the experimental protocol the rats were euthanized with CO_2 for 10 minutes and the connective tissues around the knee joints were collected.

Specimen preparation

To study the effects of diabetes on the matrix organization, right knee joints, left patellar ligaments (PL), medial collateral ligaments (MCL), and lateral collateral ligaments were maintained in neutral buffered formalin. After decalcification for 1 to 4 days with 7% nitric acid solution, the tissues were soaked for neutralization in sodium sulfate solution for a day and then washed for 10 hours under running water. Knee joint samples were divided into two parts and sectioned perpendicular to the articular surface levels. After embedding in paraffin the tissue was sliced using a microtome. From each of the portions (medial and lateral), six slices were obtained at 5 μm thickness that were spaced 50 μm apart. For PL, MCL, LCL and synovial tissue 3-4 μm thick slices were obtained. Staining was carried out using the hematoxylin/eosin (HE) double staining method. Picrosirius, prepared with 0.2% Sirius red (Direct Red 80, C. I. 35780; Aldrich, Milwaukee, WI) diluted in saturated picric acid solution, was applied to observe the structure of the collagen

fibers (Dayan et al., 1989). Right knee joint cartilage slices were stained with Safranin O/ fast green to evaluate proteoglycans.

Collagen profile area density

The histochemical characterization of the collagen was determined in synovia and cartilage from the knee joint, and in tissue specimens from PL, MCL and LCL stained with Sirius red, and analyzed under polarized light (Junqueira et al., 1982). In this method, Sirius red binds selectively to collagen, giving colored products in which the bound dye is proportional to the amount of collagen present (Junqueira et al.,1979; Kuttan and Di Ferrante, 1980; Marrotta and Martino, 1985). Quantitative studies using Sirius red may be performed through measurement of its color intensity. Color intensity is proportional to the collagen concentration and measurement of the emitted light during observation using polarized light. The birefringence also reflects the degree of parallel orientation and state of aggregation of the collagenous structures (Saldiva et al., 1989). In summary, measurements of collagen birefringence in slides stained with Sirius red reflect not only the amount of collagen, but also the macromolecular collagen arrangement in the extracellular matrix.

A systematic random sampling of ten histologic fields of view per case was used for collagen profile area density evaluation at a magnification of 400x. The collagen area was determined by optical density in the image analysis system, consisting of an Olympus camera applied to an Olympus BX-51 microscope (Center Valley, Pennsylvania). Micrographs were processed using *Image ProPlus 6.0 software* (Media Cybernetics, Inc., Bethesda, MD).

The threshold for fibers for each collagenous system was established for each slide to discern the fibers from the background of the image after enhancing the contrast to a point at which the fibers were easily identified by birefringent (collagenous) bands (Negri et al., 2000). The collagen profile area occupied by the fibers was determined by digital morphometry by adjusting the threshold level of measurement up to the (reddish-orange) density of the fibers of the collagenous system. The results are expressed as the area of the collagen fibers divided by the total area of tissue, expressed as a percentage.

Quantification of proteoglycans

Proteoglycans of the right knee joint cartilage slices were stained with Safranin O/fast green and were quantified according to the intensity of red staining using an image analysis system (*Image ProPlus 6.0 software*) as described above.

Immunofluorescence of collagen types

Longitudinal sections of knee joints, PL, MCL, and LCL prepared on slides previously treated with (3-aminopropyl)triethoxysilane (Sigma Chemical Co., St. Louis, MO) were immersed in xylene (60°C) for 20 minutes followed by two changes of cold xylene. Specimens then were hydrated by washing in successive, decreasing concentrations of ethanol (100% to 75%), distilled water and sodium phosphate buffer (PBS). For the exposure and recovery of antigenic sites, knee joint specimens were subjected to enzymatic treatment with bovine pepsin (10,000 units of enzyme/digested tissue UTD; Sigma Chemical Co., St. Louis, MO) diluted in acetic acid buffer to 0.5 M (4 mg/ml; pH 2.5) for 45 minutes at 37°C. To recover articular cartilage antigenic sites, specimens were digested with chondroitinase ABC (2 UTD/ml) diluted in Trizma-acetate buffer (0.5 M; pH 2.5) for 3 hours at 37°C. After three washes with PBS, the slices were incubated with bovine pepsin in acid buffer (4 mg/ml; pH 2.5) for 30 minutes at 37°C. All the treated sections were then washed three times for 10 minutes each with PBS, and the reaction was blocked with 5% milk in PBS for 30 minutes. Ligament and synovia slices were incubated with rabbit polyclonal anti-collagen type I (1:10 in PBS) and anti-collagen type V (1:30) antibodies and mouse monoclonal anti-collagen type III (1:20; Calbiochem Inc., San Diego, CA). Cartilage slices were incubated with rabbit polyclonal anti-collagen type II (1:200) and anti-collagen type XI (1:500) antibodies overnight at 4°C. The sections then were washed in PBS with 0.05% Tween® 20 and were then incubated for 90 minutes with rabbit or mouse anti-IgG antibody conjugated with fluorescein (FITC; Sigma Chemical Co., St. Louis, MO) diluted 1:50 in PBS and Evans blue (0.006%). Negative control reactions consisted of omitting the primary antibody step from the protocol. Sections were mounted with buffered glycerin. The reaction was evaluated using an Olympus BX-51 fluorescent microscope.

Statistical analysis

The Shapiro-Wilk test was used for normality and Levene's one-way analysis was used for homogeneity of variance. A t-test and an ANOVA with the Bonferroni post-hoc method for multiple comparisons were performed when appropriate. P values <0.05 were considered significant. Values were expressed as means ± standard deviations.

Results

Seventy days after STZ administration the rats had developed a well-established diabetic state characterized by a highly significant increase in blood glucose and plasma anti-CML concentrations compared with those in control rats. Polydipsia, polyuria, and significant impairment in weight gain were observed in all rats in the DG group (Table 1).

Left patellar, medial collateral and lateral collateral ligaments were morphologically similar to each other in the DG. Fig. 1 shows the morphological features of medial ligament tissue specimens distant from the bone

insertion stained with Picrosirius (Fig. 1A,B), quantified for collagen fibers (Fig. 1C), and immunoassayed for type I (Fig. 1D,E), type III (Fig. 1F,G), and type V (Fig. 1H,I) collagen.

Collagen fibers of the ligaments stained with Picrosirius exhibited a significant decrease in reddish-orange birefringence in DG rats compared to CG rats (Fig. 1A-C, Table 2). The DG ligaments exhibited weak green fluorescence of type I collagen fibers (Fig. 1E) and strong green fluorescence of type III (Fig. 1G) and type V (Fig. 1I) collagen fibers compared with CG ligaments (Fig. 1D,F,H), respectively. The DG ligaments were organized in a fibrillar pattern, which coincided with maintenance of their architecture observed in Picrosirius staining (Fig. 1B).

Fig. 2 shows the collagen structure of articular

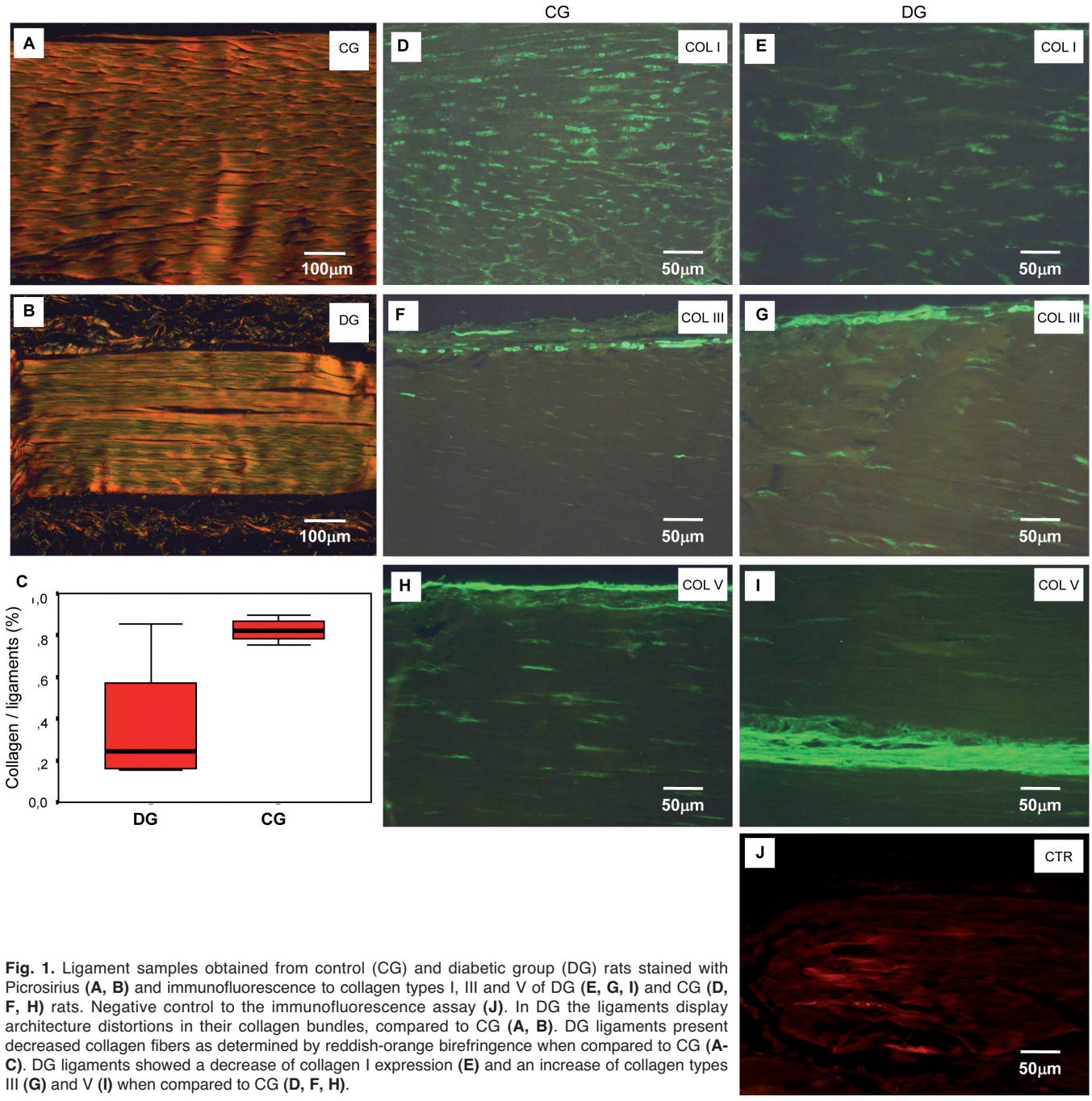

Fig. 1. Ligament samples obtained from control (CG) and diabetic group (DG) rats stained with Picrosirius **(A, B)** and immunofluorescence to collagen types I, III and V of DG **(E, G, I)** and CG **(D, F, H)** rats. Negative control to the immunofluorescence assay **(J)**. In DG the ligaments display architecture distortions in their collagen bundles, compared to CG **(A, B)**. DG ligaments present decreased collagen fibers as determined by reddish-orange birefringence when compared to CG **(A-C)**. DG ligaments showed a decrease of collagen I expression **(E)** and an increase of collagen types III **(G)** and V **(I)** when compared to CG **(D, F, H)**.

cartilage from CG and DG rats stained with Picrosirius (Fig. 2A,B), which were quantified for total collagen fibers (Fig. 2C). Collagen fibers of the articular cartilage exhibited a decrease in reddish-orange birefringence in the DG compared with the CG (Fig. 2A-C, Table 2). The immunoassay demonstrated a decrease in type II collagen expression and strong green fluorescence of type XI collagen in the DG compared with the CG (Fig. 2D-G). By applying Safranin O/fast green, the matrix staining indicated a significant decrease in proteoglycans in the DG compared with the CG (Fig. 2H-J, Table 2).

In contrast, collagen fiber remodeling quantified in

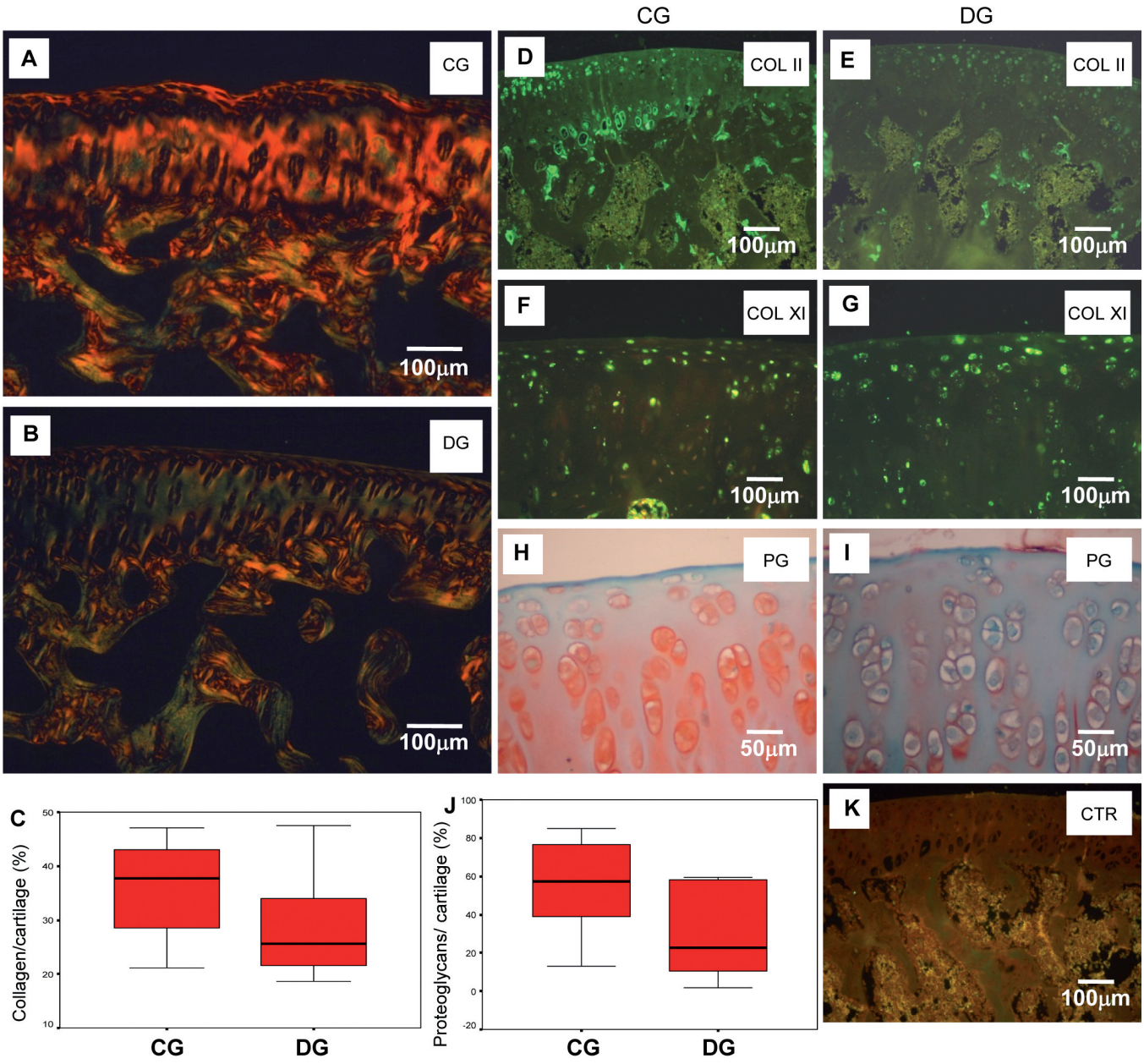

Fig. 2. Cartilage samples obtained from control (CG) and diabetic rats (DG) stained with Picrosirius **(A, B)** and immunofluorescence to types II **(D, E)** and XI **(F, G)** collagen and Safranin O/fast green staining **(H, I).** Negative control to the immunofluorescence assay **(K)**. The DG is associated with decreased collagen fibers indicated by reddish-orange birefringence in cartilage compared to CG **(A-C).** Panels **D** and **F** indicate the distribution of collagen types II and XI in the CG. DG shows a decrease of collagen II **(E)** and an increase of collagen XI **(G)** in relation to control **(D, F)**. Panel **I** demonstrates a decrease in Safranin O/fast green staining in the DG compared to the CG **(H)**, showing a significant decrease in proteoglycans in the DG compared with the CG **(J)**.

accessible synovial tissue showed an average increase in reddish-orange birefringence in the subsynovial tissue of the DG, characteristic of fibrous tissue, compared with the CG (Fig. 3A-C, Table 2). The immunoassay demonstrated an increase in type I collagen expression, reflected by intense green fluorescence on the surface and in the deep synovial region, in the DG compared with the CG group (Fig. 3D,E). Additionally, the surface and deep synovial regions exhibited a decrease in type III (Fig. 3F,G) and type V (Fig. 3H,I) collagen. Note that type III collagen expression displayed a homogeneous pattern, and type V collagen displayed a heterogeneous distribution in the subsynovial tissue after the induction of diabetes (Fig. 3G,I).

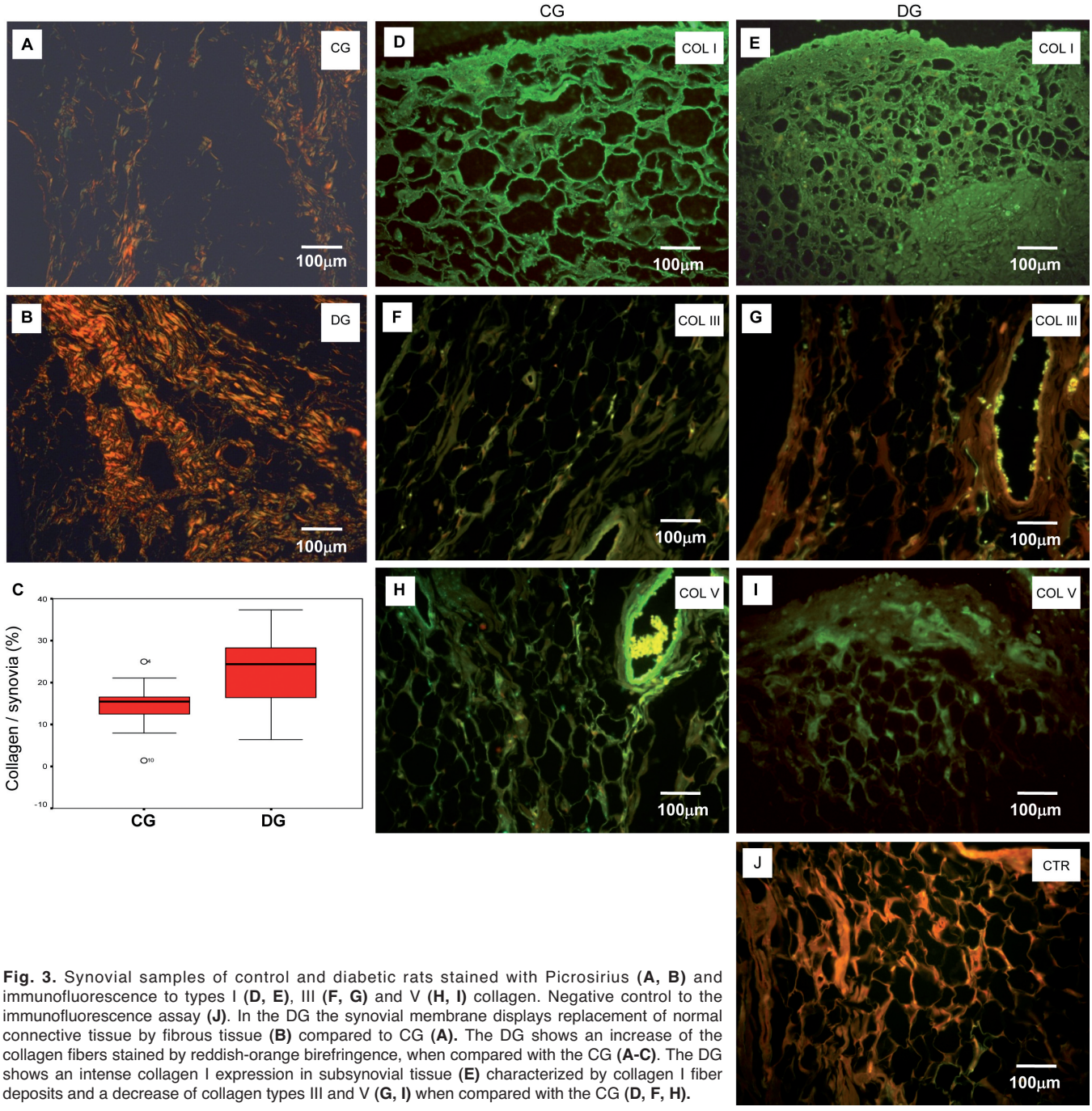

Fig. 3. Synovial samples of control and diabetic rats stained with Picrosirius **(A, B)** and immunofluorescence to types I **(D, E)**, III **(F, G)** and V **(H, I)** collagen. Negative control to the immunofluorescence assay **(J)**. In the DG the synovial membrane displays replacement of normal connective tissue by fibrous tissue **(B)** compared to CG **(A).** The DG shows an increase of the collagen fibers stained by reddish-orange birefringence, when compared with the CG **(A-C)**. The DG shows an intense collagen I expression in subsynovial tissue **(E)** characterized by collagen I fiber deposits and a decrease of collagen types III and V **(G, I)** when compared with the CG **(D, F, H).**

Table 1. General characteristics of diabetic and control rats at 1 and 70 days after streptozotocin administration.

	1 (day)	70 (days)
Body weight (g)		
Diabetic	223±26	263±19,97*
Controls	229.5±32	460.46±65.43
Blood glucose (mg/dl)		
Diabetic	75.8±7.4	377±37.53*
Controls	78.3±6.7	97.46±6.7
Plasma anti-CML (ng/ml)		
Diabetic	-	2.88±1.05*
Controls	-	1.55±0.61

*: $P<0.05$ compared to controls

Table 2. Morphometric analyses of knee articular cartilage and total collagen quantification in ligament, cartilage and synovia of diabetic and control rats.

	Articular cartilage morphometric analysis			
	Proteoglycans (%)	Total collagen quantification (%)[a]		
		Ligament	Cartilage	Synovia
Diabetic	28.91±23.38*	26.58±15.78*	28.64±8.21*	22.70±8.20*
Controls	55.48±25.94	82.04±5.05	33.05±8.53	14.45±5.39

[a]: The collagen fiber content in the ligament, cartilage, synovial areas were measured and expressed as the amount of fibers divided by the total solid parenchyma area studied, including the cellular components. The final results are expressed as the amount of collagenous system fibers (in area) per total. *: $P<0.05$ compared to controls

Discussion

The purpose of the present study was to determine the ECM histoarchitecture of ligaments, cartilage and synovial tissue in STZ-induced diabetic rats by using histochemistry, immunofluorescence and digital morphometry to obtain 2-D collagen area density. In the cartilage and ligaments of these animals we found a substantial decrease in collagen profile area densities, in addition to a decrease in cartilage proteoglycans compared to control animals. In contrast, the synovial tissue exhibited an increase in collagen profile area densities in the DG rats compared with those in the CG.

We found that ligaments in diabetic rats were characterized by decreased collagen profile area density. Furthermore, this was specifically a decrease of type I collagen, which is the most abundant collagen in ligaments. In this way, we suggest that the decrease in collagen area density may correspond to the type I collagen. On the other hand, types III and type V collagen, which are quantitatively found in minor percentages, have been found to be increased in ligaments of the DG rats (Birk and Mayne, 1997). This disorganization of collagen may provide a basis for the altered connective tissue function observed in diabetic patients (Rey et al., 2003).

In the present study, the experimental diabetes model revealed an initial fibrotic process in the ligaments. In fact, the deposition of type III collagen is a well-established phenomenon observed in the early stages of fibrosis, whereas type I collagen is the predominant collagen deposited at later stages (Kenyon et al., 2003). This fact suggests that collagen fibers can be determined by the association between different collagen types, resulting in heterotypic fibrils in which type I collagen is the main structural component. Interestingly, in ligaments we found an increase in type V collagen, which participates in fibrillogenesis by regulating the size and diameter of collagen fibers and directly influences ECM remodeling (Aydeniz et al., 2008). Our data demonstrated a higher expression of type III and V collagen and decreased expression of type I collagen in ligaments of diabetic rats, resulting in a predominance of thin collagen fibers (III/V), which could result in tissue remodeling and may lead to ligament weakness. Satomi et al. (2008) reinforce these results in human posterior tibial tendon dysfunction and suggest that these fibrils are structurally less resistant to mechanical forces.

Similar to the results in ligaments, we found that the cartilage collagen profile area density is less expressed in the DG, which by immunofluorescence was characterized as type II collagen, the main structural component in cartilage. In contrast, the type XI collagen profile area density has a higher expression in cartilage of the DG. This fact could be related to its function and similar molecular constitution to type V collagen in different connective tissues (Eyre and Wu, 1995; Gregory at al., 2000). The increase in this collagen suggests an intense remodeling of the tissue due to type XI collagen after diabetes induction, which may be reflected in the formation of a collagen pattern that is inadequate for the biomechanical requirements of cartilage.

Additionally, the decrease in cartilage proteoglycans in diabetic animals is another indication of a macromolecular change in the cartilage ECM, resulting in biomechanical changes in this tissue and consequent joint function alterations. Cartilage is an avascular tissue, and its biochemical changes may be attributable to adjacent subsynovial tissue and synovial fluid, which are responsible for cartilage nutrition (Stefek at al., 2000; Miles et al., 2005; Rey et al., 2003). Although other authors did not specifically study different collagen types, they have also found that total collagen production was decreased in bone and cartilage in STZ-induced diabetic rats (Spanheimer et al., 1988; Umpierrez et al., 1989).

The age of the animals, as well as the present results compared to normal, healthy collagen remodeling in these tissues, deserve discussion. Collagen remodeling in the context of healthy adult articular cartilage is very limited, since fibril orientation is completed at puberty in

articular cartilage. However, collagen content in this tissue does not stabilize before adulthood (Rieppo et al., 2009; Julkunen et al., 2010; van Turnhout et al., 2010). These statements lead to some questions. Are only developing tissues susceptible to diabetes-related collagen remodeling? Would similar results be expected for full grown animals and for each investigated tissue and/or collagen type? The structure and composition of articular cartilage change during development and growth, as well as in response to varying loading conditions. Therefore, the collagen fibril network of articular cartilage undergoes significant changes during maturation, which occurs earlier in tibial than in femoral cartilage and is most pronounced in the deep zone (Julkunen et al., 2009, 2010; Rieppo et al., 2009). These changes modulate the functional properties of cartilage. In the perinatal cartilage, the predominant collagen orientation is parallel to the articular surface throughout the tissue depth. However, this remodels to the Benninghoff structure, with predominantly perpendicular arcades, before an individual reaches sexual maturity (van Turnhout et al., 2010). In our experimental model, the rats had two months at the time of diabetes induction, which represents the period of the life of a young rat. Moreover, rats reach puberty (sexual maturity) at 40 days. During this period, the cartilage collagen network architecture represents the classic Benninghoff structure, but the collagen content increases into adulthood. In our experimental protocol, diabetic and healthy rats were sacrificed at four months of age, which is considered to be adulthood. In this period, theoretically both diabetic and control rats possess the same collagen network architecture orientation. However, the control group has the normal collagen composition, which is formed largely by collagen type II and about 5-10% collagen type XI, which primarily regulates the diameter of very thin collagen fibrils in cartilage. In contrast, the cartilage of the diabetic animals presented a decrease in collagen type II and an increase in collagen type XI, which may reflect the formation of a fibrillar network that is inadequate for the biomechanical requirements of cartilage. It is well known that collagen remodeling in healthy adult articular cartilage is very limited. Therefore, in comparing the results of collagen in cartilage from control and diabetic rats, we can assume that the changes in the collagen network are due to the diabetes process induced by STZ. It is well known that advanced glycation end products (AGE) can be formed in the late stage of the glycation reaction in diabetes, inducing collagen crosslinkages and altering the solubility and proteolytic digestion of collagen (Semba et al., 2010). These effects are manifested as stiffness in human skeletal muscles and articular cartilage (Semba et al., 2010). Although in our protocol collagen remodeling was analyzed between young-age and adult rats, we believe that the alterations in collagen types found in diabetic animal tissues were due the increase in blood glucose. We would expect to observe similar results in full grown animals.

Surprisingly, in synovial tissue, we observed an increase in collagen profile area densities, a histological pattern that was completely different from the other joint components. The primary function of the synovia is the secretion of synovial fluid that lubricates and nourishes articular cartilage (McGonagle et al., 2010). Thus, in experimental diabetes models these functions can be compromised, contributing to articular pathology. Additionally, we found higher levels of plasma anti-carboxymethyllysine in an experimental diabetes model that might influence the phenotypic modification of synoviocytes in cell growth and the expression of inflammatory markers and osteogenesis, resulting in the stimulation of collagen synthesis. This may also explain the prevalence of structural joint impairments in diabetic patients (Arkkila et al., 2003).

Thus, the collagen profile area densities decreased in the cartilage and ligaments, and increased in synovial tissue, which may be primary events related to diabetes. Regardless of the mechanism, diabetic rats exhibited prominent articular component remodeling. This finding may contribute to the understanding of the functional limitations of diabetic patients and the association of diabetes with rheumatic diseases. Finally, these results may have an important role in the development of new therapies for diabetes, which could have a major impact on the quality of life of patients affected by this disease.

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References

Arkkila P.E., Koskinen P.J., Kantola I.M., Rönnemaa T., Seppänen E. and Viikari J.S. (2003). Biochemical markers of types I and III collagen and limited joint mobility in type 1 diabetic patients. Acta Diabetol. 40, 151-155.

Aydeniz A., Gursoy S. and Guney E. (2008). Which musculoskeletal complications are most frequently seen in type 2 diabetes mellitus? J. Int. Med. Res. 36, 505-511.

Birk D.E. and Mayne R. (1997). Localization of collagen type I, III and V during tendon development. Changes in collagen type I and III are correlated with changes in fibril diameter. Eur. J. Cell Biol. 72, 352-361.

Bolzán A.D. and Bianchi M.S. (2002). Genotoxicity of streptozotocin. Mutat. Res. 512(2-3), 121-134.

Brennan M. (1989). Changes in solubility, non-enzymatic glycation, and fluorescence of collagen in tail tendons from diabetic rats. J. Biol. Chem. 264, 20947-20952.

Burner T.W. and Rosenthal A.K. (2009). Diabetes and rheumatic diseases. Curr. Opin. Rheumatol. 21, 50-54.

Chbinou N. and Frenette J. (2004). Insulin-dependent diabetes impairs the inflammatory response and delays angiogenesis following Achilles tendon injury. Am. J. Physiol. Regul. Integr. Comp. Physiol. 286, R952-R957.

Dayan D., Hiss Y., Hirshberg A., Bubis J.J. and Woiman M. (1989). Are the polarization colors of Picrosirius red-stained collagen determined only by the diameter of the fibers? Histochemistry 93, 27-29.

Eyre D.R. and Wu J-J. (1995). Collagen structure and cartilage matrix integrity. J. Rheumatol. 22, 82-85.

Fujinaka Y. (2009). Diabetic osteoarthropathy. Clin. Calcium 19, 1299-1303.

Gregory K., Oxford J.T., Chen Y., (2000). Structural Organization of distinct domains within the non-collagenous N-terminal region of collagen type XI. J. Biol. Chem. 275, 11498-11506.

Julkunen P., Harjula T., Iivarinen J., Marjanen J., Seppänen K., Närhi T., Arokoski J., Lammi M.J., Brama P.A., Jurvelin J.S. and Helminen H.J. (2009). Biomechanical, biochemical and structural correlations in immature and mature rabbit articular cartilage. Osteoarthritis Cartilage 17, 1628-1638.

Julkunen P., Iivarinen J., Brama P.A., Arokoski J., Jurvelin J.S., Helminen H.J. (2010). Maturation of collagen fibril network structure in tibial and femoral cartilage of rabbits. Osteoarthritis Cartilage 18, 406-415.

Junqueira L.C.U., Bignolas C. and Brentani R.R. (1979). A simple and sensitive method for the quantitative estimation of collagen. Anal Biochem. 94, 96-99.

Junqueira L.C.U., Montes G.S. and Sanchez E.M. (1982). The influence of tissue section thickness on the study of collagen by the picrosirius polarization method. Histochemistry 74, 153-156.

Kameyama M., Meguro S., Funae O., Atsumi Y. and Ikegami H. (2009). The presence of limited joint mobility is significantly associated with multiple digit involvement by stenosing flexor tenosynovitis in diabetics. J. Rheumatol. 36, 1686-1690.

Kenyon N.J., Ward R.W., McGrew G. and Last J.A. (2003). TGF-ß1 causes airway fibrosis and increased collagen I and III mRNA in mice. Thorax 58, 772-777.

Kuttan R. and Di Ferrante N. (1980). Sirius red-collagen interaction: a method for the measurement of collagen and bacterial collagenase activity. Biochem. Int. 1, 455-462.

Le Pape A., Muh P. and Bailey A.J. (1981). Characterization of N-glycosylated type I collagen in streptozotocin-induced diabetes. Biochem. J. 197, 405-412.

Linsenmayer T.F. (1991). Collagen. In: Cell biology of extracellular matrix. 2nd ed. Hay E.D. (ed). Plenum Press. New York. pp 7-43.

Marotta M. and Martino G. (1985). Sensitive spectophotometer method for the quantitative estimation of collagen. Anal. Biochem. 150, 86-90.

McGonagle D., Tan A.L., Carey J. and Benjamin M. (2010). The anatomical basis for a novel classification of osteoarthritis and allied disorders. J. Anat. 216, 279-291.

Miles C.A., Avery N.C., Rodin V.V. and Bailey A.J. (2005). The increase in denaturation temperature following cross-linking of collagen is caused by dehydration of the fibres. J. Mol. Biol. 346, 551-556.

Negri E.M., Montes G.S., Saldiva P.H. and Capelozzi V.L. (2000). Architectural remodelling in acute and chronic interstitial lung disease: fibrosis or fibroelastosis? Histopathology 37, 393-401.

Novelli M., Fabregat M.E., Fernandez-Alvarez J., Gomis R., and Masiello P. (2001). Metabolic and functional studies on isolated islets in a new rat model of type 2 diabetes. Mol. Cell Endocrinol. 175, 57-66.

Rey L.D., Della C.R., Mukai M., Silva M.B. and Skare T.L. (2003). Diabetes: tendinitis and enthesophathy. Rev. Bras. Reumatol. 43, 218-222.

Rieppo J., Hyttinen M.M., Halmesmaki E., Ruotsalainen H., Vasara A., Kiviranta I., Jurvelin J.S. and Helminen H.J. (2009). Changes in spatial collagen content and collagen network architecture in porcine articular cartilage during growth and maturation. Osteoarthritis Cartilage 17, 448-55.

Saldiva P.H., Delmonte V.C., Carvalho C.R., Kairalla R.A. and Auler Júnior J.O. (1989). Histochemical evaluation of lung collagen content in acute and chronic interstitial diseases. Chest 95, 953-957.

Sarkar P., Pain S., Sarkar R.N., Ghosal R., Mandal S.K. and Banerjee R. (2008). Rheumatological manifestations in diabetes mellitus. J. Indian. Med. Assoc. 106, 593-594.

Satomi E., Teodoro W.R., Parra E.R., Fernandes T.D., Velosa A.P., Capelozzi V.L. and and Yoshinari N.H. (2008). Changes in histoanatomical distribution of types I, III and V collagen promote adaptative remodeling in posterior tibial tendon rupture. Clinics 63, 9-14.

Semba R.D., Bandinelli S., Sun K., Guralnik J.M. and Ferrucci L. (2010). Relationship of an advanced glycation end product, plasma carboxymethyl-lysine, with slow walking speed in older adults: the In CHIANTI study. Eur. J. Appl. Physiol. 108, 191-195.

Spanheimer R.G., Umpierrez G.E. and Stumpf V. (1988). Decreased collagen production in diabetic rats. Diabetes 37, 371-376.

Stefek M., Gajdosik A., Gajdosikova A. and Krizanova L. (2000). p-Dimethylaminobenzaldehyde-reactive substances in tail tendon collagen of streptozotocin-diabetic rats: temporal relation to biomechanical properties and advanced glycation endproduct (AGE)-related fluorescence. Biochim. Biophys. Acta 1502, 398-404.

Teodoro W.R., Velosa A.P., Witzel S.S., Garippo A.L., Farhat C., Parra E.R., Sonohara S., Capelozzi V.L. and Yoshinari N.H. (2004). Architectural remodelling in lungs of rabbits induced by Collagen V immunization. A preliminary morphologic model to study diffuse connective tissue diseases. Pathol. Res. Pract. 200, 681-691.

Tonra J.R., Cliffer K.D., Carson S.R., Lindsay R.M., Bodine S.C. and DiStefano P.S. (2001). Reduced Ia-afferent-mediated Hoffman reflex in streptozotocin-induced diabetic rats. Exp. Neurol. 172, 220-227.

Turk Z., Misur I., Turk N. and Benko B. (1999). Rat tissue collagen modified by advanced glycation. Correlation with duration of diabetes and glycemic control. Clin. Chem. Lab. Med. 37, 813-820.

Umpierrez G.E., Goldstein S., Phillips L.S. and Spanheimer R.G. (1989). Nutritional and hormonal regulation of articular collagen production in diabetic animals. Diabetes 38, 758-63.

van Turnhout M.C., Schipper H., Engel B., Buist W., Kranenbarg S. and van Leeuwen J.L. (2010). Postnatal development of collagen structure in ovine articular cartilage. BMC Dev. Biol. 10, 62.

Wang H., Layton B.E. and Sastry A.M. (2003). Nerve collagens from diabetic and nondiabetic Sprague-Dawley and biobreeding rats. An atomic force microscopy study. Diabetes Metab. Res. Rev. 19, 288-298.

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