Good glycaemic control reduces oxidation and glycation end-products in collagen of diabetic rats

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Summary Blood glucose control plays a prominent role in the aetiology of diabetic complications. Recent data support the hypothesis that non-enzymatic pathways (glycation and oxidation) are involved in the pathogenesis of tissue damage in diabetes mellitus. In this study the level of pentosidine, a marker of glycation, and the intensity of collagen-linked fluorescence glycation (370/440 and 335/385 nm) and oxidation-related (356/460 and 390/460 nm), have been examined in spontaneously diabetic rats with good and poor glycaemic control. Pentosidine increased dramatically in rats with poor control, and slightly in those with good control. At the end of the study, after 6 months of diabetes, pentosidine levels were 13 ± 5 and 2.1 ± 0.5 pmol/mg collagen, respectively (control rats: 1.1 ± 0.1 pmol/mg collagen). A similar pattern was observed for both glycation or oxidation-related fluorescence. The group of rats with poor control always showed elevated average values when compared to rats with good control, with a relative increase of over 200 %. The results emphasize the role

of good glycaemic control in preventing the growth of glycation or oxidation end-products in collagen. On comparison between the general mean level of all glycated haemoglobin and the mean pentosidine level of the three groups, a very good exponential correlation was found ($r = 0.993$, $p < 0.001$). The fluorescence values presented a less strong relationship, but a correlation with glycaemic control was still present. If the post-translational modifications of proteins play a leading role in the pathogenesis of complications it is possible to conclude that strict glycaemic control, obtained by accurate insulin therapy can prevent them by inhibiting the non-enzymatic modification of proteins and delaying their accumulation in collagen. The therapeutic implications are obvious. [Diabetologia (1996) 39: 1440–1447]

Keywords Glycation, oxidation, advanced glycation end products, pentosidine, fluorescence, collagen, non-enzymatic modification, glycaemic control, protein adducts.

The recently concluded Diabetes Control and Complications Trial [1] is a milestone in understanding the development of complications in insulin-dependent diabetes mellitus. Patients followed over 6 years

showed a lower incidence of microvascular and neurological complications with a better glycaemic control. The conclusion of this large longitudinal study is that strict glycaemic control is the best way to delay the onset of complications, focusing on the importance of hyperglycaemia in the pathogenic mechanism [1]. The perturbation induced by hyperglycaemia leads to damage through several insulin-independent enzymatic pathways or by spontaneous post-synthetic protein modifications [2, 3]. Non-enzymatic glycation gives rise over time to a series of irreversible compounds with the formation of cross-links in extracellular matrix, alterations in cells and in nucleic acids [4, 5], and generates reactive free radicals

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Abbreviations: ND, Non-diabetic; IT, intensive therapy; CT, conventional therapy; GHb, glycated haemoglobin; HPLC, high pressure liquid chromatography; MDA, malondialdehyde; HNE, hydroxynonenal.

through auto-oxidation of Amadori product and/or glycated collagen [6, 7]. Furthermore by-products (aldehydes) of lipid oxidation, binding amino or sulfhydryl groups [8, 9], are also able to form adducts with proteins, contributing to the tissue damage.

A clear interconnection exists between glycation and oxidation [10–17]. There are two general hypotheses on the importance of these modifications: 1) a prevailing role of auto-oxidation as a leading factor in the pathogenesis of conformational changes, breaks, cross-links and formation of fluorescent end-products [12, 16]; 2) the prevalence of glucose and other molecules as protein modifiers, with the oxidation steps thought to be collateral contributors of a main event, the Maillard reaction [14, 15].

Several strong experimental studies indicate a role for non-enzymatic modifications in the development of diabetic sequelae [18–22]; however, a restricted number of *in vivo* studies have been performed to prove this relationship and study the effect of good glycaemic control, as the number of available markers is limited [23–25].

Since both glycation end-products and protein modifications by lipoperoxidation by-products are fluorescent [26–29] it is possible to take advantage of this characteristic to measure these products. Moreover a specific assay has been developed recently to quantify the concentration of pentosidine, a well characterized advanced glycation end-product [30].

This work evaluates the level of glycation and lipoperoxidation fluorescent compounds and the pentosidine content in subcutaneous collagen of BB/Wor diabetic rats, a strain considered an excellent animal model for human insulin-dependent diabetes. The effect of intensive metabolic control in protection against the formation and accumulation of glycation and lipoperoxidation derived compounds has been also examined.

Materials and methods

Animals. Thirty diabetes-prone BB/Wor/Mol/BB male rats purchased from Møllegaard Ltd. (Skensved, Denmark) were randomly caged in groups of two and fed with standard diet (Piccioni Spa, Milan, Italy). Diabetes resistant BB/Wor/Mol/WB rats were used as control group ($n = 12$).

The animals were housed until the programmed age for study was reached (1, 3, and 6 months after the onset of diabetes). All rats were maintained at 22–24 °C with 12 h dark/light cycle, following common procedures for good animal care. Blood glucose was determined with reactive strips (Haemoglucotest; Boehringer Mannheim Italia Spa, Milan, Italy) and read with reflectometer (Reflolux S; Boehringer Mannheim) three times per week by drawing blood from a tail vein.

Glycated haemoglobin (GHb) levels were measured monthly by affinity chromatography (Glycaffin; Isolab, Akron, Ohio, USA). A blood glucose over 11 mmol/l was considered indicative of diabetes onset, which occurred at 8–12 weeks of age.

Once diabetes was confirmed (two consecutive hyperglycaemic values) insulin therapy was started. A subcutaneous slow-release implant of stearate insulin (Limplant; Linshin Inc., Canada) was used, adjusting the number of implants to achieve a good or fair-to-poor glycaemic control.

Diabetic rats were randomly divided into two groups: one group intensively treated with insulin, whose blood glucose was maintained under 11 mmol/l (IT) and one with a conventional standardized insulin therapy, whose blood glucose was often beyond 16 mmol/l (CT). The first group received 1.5 times more insulin than the second group.

Sample preparation. As programmed for diabetes duration, animals were killed after anaesthesia with intraperitoneal injection of sodium pentobarbital (65 mg/kg). Blood was drawn from the inferior vena cava and aliquots were used for the last measurement of glucose and GHb. A midline incision in the skin of the abdomen was made and a sample of subcutaneous connective tissue was removed. All fragments of muscular tissue were excised from connective tissue. The samples were washed thoroughly in normotonic saline solution and were frozen and stored at –80 °C until used for measurements. A small amount of collagen (20 mg) was finely minced and homogenized in ice-cold phosphate buffer saline 0.1 mol/l (pH 7.4) with a Polytron homogenizer (Kinematica, Lucerne, Switzerland), set at level 3 for 2–3 min. Lipids were extracted from the tissue with chloroform/methanol (2:1) by mild shaking overnight. The pellets were washed with methanol and water and finally stored overnight at 4 °C in 0.05 mol/l Tris-HCl buffer (pH 7.4) with 1 mol/l NaCl and freed from soluble protein by washing with HPLC-grade water. The remaining tissue was digested for 24 h (at 37 °C with mild shaking) with purified collagenase (type VII, Sigma, St. Louis, Mo., USA) in HEPES buffer (0.02 mol/l, pH 7.5) containing 0.01 mol/l CaCl_2 . The supernatant was used for the determination of hydroxyproline content and fluorescence. The undigested collagen amounted to less than 20 % of the total.

End-product determination. Fluorescence intensity was determined in the digests with a L55B Perkin-Elmer (Verkauf GmbH, Munchen, Germany) spectrophotofluorometer at: a) 440 nm upon excitation of 370 nm for Maillard products related fluorescence [31]; b) 335 nm excitation and 385 nm emission for pentosidine-like products [32]; c) 460 nm upon excitation of 390 nm and of 356 nm, which are the specific wavelengths for malondialdehyde (MDA) and hydroxynonenal (HNE) protein-adducts [33]. A hydroxyproline assay was performed according to the method of Stegemann and Stalder [34] to give relative collagen content. The intensity of fluorescence was expressed in arbitrary units of fluorescence (AUF) per milligram of hydroxyproline. Twenty mg of subcutaneous collagen was used for pentosidine quantitation [30]. After homogenization and delipidization, the pellets, washed at least twice with phosphate buffer (0.1 mol/l, pH 7.4), were hydrolysed in 2 ml of HCl 6N, under N_2 atmosphere for 24 h at 110 °C in borosilicate tubes with a screw cap. After hydrolysis, acid was evaporated with Modulyo lyophilizer (Edwards Alto Vuoto S.p.A., Milan, Italy) and each sample was reconstituted in water containing 0.01 mol/l heptafluorobutyric acid (Pierce, Prodotti Gianni, Milan, Italy) and filtered through 0.45 μm pore diameter filter (Ultrafree MC, Millipore, Milan, Italy).

Pentosidine was determined in reconstituted samples by reverse phase chromatography (25 $\times$ 0.46 cm Vydac type 218TP (10 μm) C-18 column, Separation Group, Hesperia, Calif., USA) on a Waters HPLC system (WAT717 as autosampler, WAT510 as pump and an NEC 486 Image computer as

controller, Waters, Milan, Italy). "Millenium software" was used to manage the chromatography.

Separations were made with a linear gradient program of 10–17% acetonitrile from 0 to 22 min, containing water (MilliQ, Millipore) and heptafluorobutyric acid (0.01 mol/l) as a counter ion [30]. The effluent was scanned by a 470 Waters fluorescent detector set at 385 nm emission upon 335 nm excitation wavelength. Attenuation was fixed at 4, gain at $\times 100$ and filter at 1.5 s. The pentosidine area was collected, dried and resuspended in 0.02 mol/l sodium acetate buffer (pH 4.7) for a further separation, since pentosidine peak was not well resolved. The collected fraction was loaded onto a cation exchange column (SP-5PW Waters) with a mobile phase, characterized by a curvilinear gradient of NaCl from 0 to 0.06 mol/l (from 0 to 40 min) in sodium acetate 0.02 mol/l (pH 4.5) [30]. The fluorimeter was set as previously reported for the reverse phase column. A pentosidine synthetic standard was injected at the beginning of every run to quantitate the level in the sample by peak area comparison.

Statistical evaluation. Analysis of variance and Tukey-Kramer post-test with multiple comparisons were used. Analysis of linear or non-linear regression and correspondent coefficient of correlation was performed to assess the fit between the two parameters. The fluorescence values were normalized for age and duration of diabetes as previously described [35]. Briefly adjustment was made using the graph co-ordinates of variables and the slope of the computed regression line of non-diabetic rats.

A two-tail p value was considered significant if less than 0.05. Data are expressed as mean $\pm$ SEM.

Results

Rat growth was regular until the onset of diabetes; diabetic animals gained weight slower than controls: after 24 weeks of diabetes the body weight was lower than in control (ND) animals (Fig. 1). However, the group submitted to intensive insulin therapy (IT) showed a more regular body weight gain than the group on conventional therapy (CT). After onset of diabetes blood glucose sharply increased. In the CT group blood glucose rapidly reached 20 mmol/l and was only partially corrected by the therapy; GHb gradually achieved levels over 8%, but sometimes peaked at 14% (ND: $n = 54$, $4.2 \pm 0.1\%$ and CT: $n = 90$, $8.8 \pm 0.3\%$). In the IT group the glucose level remained beyond normal concentrations but only occasionally exceeded 12 mmol/l; moreover rats seldom presented hypoglycaemia (blood glucose lower than 2.5 mmol/l); GHb ranged between 4 and 8% with an overall average of $6.0 \pm 0.1\%$ ($n = 90$) (ANOVA: $F = 93.3$, $p < 0.001$; ND vs IT, ND vs CT and CT vs IT, all $p < 0.001$) (Fig. 2).

Raw tissue fluorescence in non-diabetic rats was similar to that obtained in another strain of non-diabetic rats, in which a slow initial increase was observed during youth and middle age [17]: both collagen glycation and oxidation-related fluorescence intensities increased slightly during the observation period, the 330 nm/420 nm and 356 nm/460 nm fluorescence

Fig. 1. Increase of body weight in control (○), diabetic with good (IT △) or poor (CT ▼) glycaemic control rats

Fig. 2. Glycated haemoglobin levels during the observation period. Bars represent mean $\pm$ SEM; non-diabetic (ND □); diabetic in intensive insulin therapy (IT ▨); diabetic in conventional therapy (CT ■); months of diabetes duration in brackets. * $p < 0.05$ vs correspondent basal value

showed the most increase (Fig. 3). The 370/440 nm fluorescence is usually related to general advanced glycation end-products and the 335/385 nm fluorescence to pentosidine-like compounds, since pentosidine represents only 30% of this fluorescence [33, 34]. A weak increase was observed in the ND group, while in both diabetic groups the fluorescence values distinctly rose. However, in the IT group, though the 370/440 nm fluorescence was higher than in ND, it remained steady over time, while in the CT group it had more than doubled after 1 month of diabetes and had further increased at 6 months of diabetes. On the other hand the 335/385 nm fluorescence showed an apparently slower increase after the onset of diabetes, but a clear increase was noticeable during the observation. The level in the CT group was higher than in the IT group (Table 1) and at the end of the study it was three times higher than at the starting level.

Both wavelengths of oxidation-related fluorescence showed an increase with aging in the ND rats.

Table 1. Analysis of variance (F) and post-tests (Tukey-Kramer) in non-diabetic (ND), diabetic in intensive (IT) and conventional (CT) insulin therapy

Fluorescence (excitation nm/ emission nm)	Age (months)	F	ND vs IT	ND vs CT	IT vs CT
370 nm/440 nm	4	6.2 ^a	NS	< 0.05	NS
	6	11.3 ^b	NS	< 0.01	< 0.05
	9	32.9 ^c	NS	< 0.001	< 0.001
335 nm/385 nm	4	3.8	NS	NS	NS
	6	26.5 ^c	< 0.05	< 0.001	< 0.01
	9	25.5 ^c	< 0.05	< 0.001	< 0.01
356 nm/460 nm	4	19.4 ^c	NS	< 0.001	< 0.01
	6	19.5 ^c	NS	< 0.001	< 0.01
	9	19.8 ^c	NS	< 0.001	< 0.01
390 nm/460 nm	4	3.0	NS	NS	NS
	6	1.4	NS	NS	NS
	9	8.7 ^b	NS	< 0.05	< 0.05
330 nm/420 nm	4	19.1 ^c	NS	< 0.001	< 0.01
	6	19.0 ^c	NS	< 0.01	< 0.001
	9	41.7 ^c	NS	< 0.001	< 0.001

^a $p < 0.05$, ^b $p < 0.01$, ^c $p < 0.001$

After the onset of diabetes the 356/460 nm fluorescence (HNE-protein related) underwent a moderate, but significant increase if the glycaemic control was good (IT), a dramatic increase was evident if the blood glucose was poorly controlled (CT). A different pattern was exhibited by the MDA-related fluorescence (390/460 nm), indeed only at 6 months of diabetes a significant higher level was reported for the CT group (Fig. 3, Table 1).

In order to attain a more complete profile, maps of collagen linked fluorescence were explored by sequential evaluation of fluorescence spectra for each collagen sample. An evident maximum was found around 330/420 nm. A very slight increase in 330/420 fluorescence was detected in the non-diabetic and in the IT group; whereas in the CT group an early and significant increase was observed after 1 month of diabetes, the increase being even greater at the end of the study; interestingly, the IT group levels were never significantly different from the ND levels (Fig. 4, Table 1).

The comparison of the three groups of rats with each other at 1, 3 and 6 months indicated that intensive insulin treatment had noteworthy consequences on fluorescence accumulation (Table 1). At 1, 3 and 6 months of diabetes, all fluorescence levels in the IT group were not significantly higher than in similar aged NDs, the only exception being the 335 nm/385 nm fluorescence, higher at 3 and 6 months ($p < 0.05$). Treatment efficacy was still more evident comparing CTs with ITs and NDs. A clear-cut difference was found for each fluorescence and at each step studied (Table 1). The few differences between the non-diabetic group and diabetic rats in intensive treatment showed an efficient inhibition on non-enzymatic

reactions by good control. Pentosidine, a sensitive marker of glycation, showed a similar course: all groups, at the end of the study, had significantly higher values in comparison with the concentration observed during the first month of diabetes (corresponding to 4 months of age for the ND group; Fig. 3). Pentosidine content in IT rats was roughly superimposable on that of the control group at 1, 3 and even after 6 months of diabetes; at 6 months the CT group was at the highest levels, significantly more elevated than ND and IT groups (1.1 ± 0.2 [ND], 2.1 ± 0.5 [IT] and 13 ± 5 [CT] pmol/mg collagen; ANOVA $F = 6.9$; ND vs CT, $p < 0.05$ and IT vs CT, $p < 0.05$).

A study of the relationships between glycation- or oxidation-related fluorescence, pentosidine and the glucose control parameters (GHb and blood glucose) was performed. As expected, a significant coefficient of correlation was found between GHb and the mean of all previous blood glucose determinations ($n = 142$; $y = 2.29 + 1.11x$, $r = 0.639$, $p < 0.001$; Fig. 4). The mean of all blood glucose determinations before killing rats was correlated with tissue pentosidine with a non-linear correlation ($n = 30$; $y = 1.5 + 0.41x + 0.012x^2$; $r = 0.618$, $p < 0.01$). When the mean of GHb at different ages in the three groups was correlated with pentosidine it exhibited a striking non-linear regression ($y = 0.013 * e^{0.037x}$; $r = 0.993$; $p < 0.001$; Fig. 4). Significant correlations were also observed between fluorescence values and average of GHb: the strongest correlation coefficient was found with the 390 nm/460 nm fluorescence (Table 2). Even stronger correlations of fluorescence with pentosidine were found, particularly with the 370 nm/440 nm and with 335 nm/385 nm fluorescence (Table 2). In order to avoid the effect caused by possible confounding factors we adjusted the value of pentosidine for age and duration of diabetes. As expected, the age factor influenced the accumulation of glycation end-products, but the diabetes-induced differences were still present and the correlation coefficients basically not affected.

Discussion

Non-enzymatic modification of collagen is a well recognized feature of diabetes and is implicated in the pathogenesis of complications. There are no doubts that non-enzymatic glycation and oxidative stress are important reactions in the damage of collagen protein in diabetes [4–6, 8, 11, 13–25], but little is known about the kinetics of collagen after non-enzymatic modification. Early glycation (exosyllysine) is directly related to the ambient glucose concentration, but its accumulation in blood and tissues is saturable [36, 37]. A specific enzymatic degradation of Amadori product has been reported recently, but the influence of this pathway does not appear considerable [38, 39]. Conversely the amount of irreversible

Fig 3. Effect of good or poor glycaemic control on the fluorescence and pentosidine level in subcutaneous collagen of diabetic rats. Animals were killed after 1, 3, and 6 months of diabetes; at the time non-diabetic rats of the same age were also killed. Bars represent mean $\pm$ SEM; non-diabetic ($\square$ ND, $n = 12$); diabetic in intensive insulin therapy ($\square$ IT, $n = 15$); diabetic in conventional therapy ($\blacksquare$ CT, $n = 15$); AUF, Arbitrary Units of fluorescence. Months of diabetes duration in brackets * $p < 0.05$ vs correspondent basal value

end-products depends mainly, but not only [40, 41], on the degree of hyperglycaemia, giving rise to irreversible and fluorescent or non-fluorescent final products. Enzymatic degradation for advanced glycation end product (AGE) reactive intermediates has been postulated [42], but it is certainly slow and difficult; AGE removal from tissue is largely hypothetical. Thus, the pool of collagen modified by glycation and oxidation products may be considered almost insoluble, with a negligible turnover; therefore their tissue growth is not easily restricted.

These observations explain the observed age-related accumulation and sharp, impressive acceleration

that occurred with diabetes. This study confirms that diabetes induces an increase of AGE in collagen, evaluated by means of fluorescence and pentosidine.

Increased attention has been directed toward oxidative stress and related events, raising new speculative hypotheses of tissue damage [7, 8, 16, 28, 43–47]. Glycation related fluorescence showed a linear increase in intensity after the onset of diabetes, especially in the CT group, whereas the oxidation-related fluorescence manifested a time gap, but it was particularly sensitive to poor glycaemic control. Thus, diabetes induced a marked increase of fluorescence intensity at all examined wavelengths. However, in the IT group, after a slight increase, the level remained steadier, throughout the study, than in the CT group, where, as expected, an impressive effect was detected after 3 and 6 months of diabetes (Fig. 3).

The present data indicate an increase in fluorescence corresponding to glycation and in fluorescence related to the lipoperoxidation adducts. Thus, it appears that non-enzymatic glycation and oxidation are strictly connected and both contribute to the modification of biological structures; in vitro studies have provided firm evidence in this direction [6, 11,

Fig. 4 Upper panel, blood glucose (mmol/l) vs glycated haemoglobin (%) of non-diabetic and diabetic rats. Blood glucose values are the averages of all blood glucose determinations before the value of glycated haemoglobin ($n = 142$, $y = 2.29 + 1.11x$, $r = 0.639$, $p < 0.001$). Lower panel, non-linear correlation between the averages of pentosidine (pmol/mg collagen) at 1, 3 and 6 months of diabetes and the averages of all glycated haemoglobin (%) determinations before killing in non-diabetic (ND ○), diabetic in good (IT △) and poor (CT ▼) glycaemic control rats ($n = 6$; $y = 0.013 \cdot e^{0.037x}$; $r = 0.993$; $p < 0.001$)

13, 14, 16]. A strong correlation was noticed between the glycation and the oxidation-related fluorescence, this was expected as we had already found the same relationship during aging [17].

What was surprising was the correlation found between the oxidation parameters and glycaemic control, suggesting a direct relationship between worsening of glucose control and the formation of protein oxidation adducts. We are not aware of any existent contribution on this issue in the literature – in vivo at least. In the previous studies a fair or poor correlation was found between glycation related fluorescence, pentosidine and glycaemic control [18–25]. For the elaboration of our data we used the average of all the available GHb values of the antecedent period and the result was a striking significant correlation (Fig. 4). The average of GHb values and pentosidine

Table 2. Coefficients of correlation between fluorescence levels and glycated haemoglobin or pentosidine

	Fluorescence (excitation nm/emission nm)				
	370/440	335/385	356/460	390/460	330/420
Glycated haemoglobin	0.46 ^b	0.48 ^b	0.45 ^b	0.50 ^b	0.44 ^b
Pentosidine	0.83 ^c	0.80 ^c	0.71 ^c	0.67 ^c	0.72 ^c

^b $p < 0.01$, ^c $p < 0.001$

showed an exponential correlation, suggesting that the level of this AGE marker may reflect the glycaemic level over an extended period of time, longer than that recognized by markers of early phases of glycation (GHb). Further, the shape of the exponential curve is easily explicable: the early glycation, i.e. formation of the Amadori product, is a saturable reaction and no more than 15–18% of haemoglobin can be modified even during a prolonged and chronic hyperglycaemia. Conversely, pentosidine does not appear to reach saturation, or, at least, may have a much higher level of tissue saturation, continuing to accumulate in skin collagen. It is possible that the accumulation of AGE might be the direct effect of chronic hyperglycaemia: increasing the level of blood glucose increases accumulation.

Moreover, the disposal mechanism of AGE [48, 49] is likely slow or not expandable and in the face of a higher concentration of glucose and other precursors the removal may not be active enough to prevent the tissue accumulation.

A linear correlation was observed between GHb and the collagen oxidation-related fluorescence. The strict relationships found confirm that blood glucose level is directly implicated in the genesis of the fluorescent molecules derived from the two pathways (glycation and oxidation). We demonstrated that an early start in treatment makes it possible to slow down non-enzymatic glycation and oxidative stress, both activated by poor glycaemic control.

Previous studies showed a correlation between collagen post-translational modifications and presence of diabetic complications [18–22]. However, other studies have reported difficulties in repairing the damage produced in collagen. In human diabetic patients, after 1–3 years, the most favourable glycaemic control obtained a reduction as small as 10% of collagen fluorescent products [23]. In other studies, after 4 months and after 1.5 years of good glycaemic control, no verifiable changes were reported [24, 25]. Moreover, a collateral DCCT study found that intensive treatment led to collagen-linked fluorescence and pentosidine lower than that of patients on conventional treatment [50]. In our study the difference between control rats and diabetic rats with good control, initiated early, was low and not significant, whereas the CT group showed dramatically higher values in all markers. Consequently, intensive treatment must

be initiated as early as possible and maintained to achieve the lowest level of AGE, which, on the basis of recent evidence, means a low degree of complications.

These patient studies suggest that once the tissue is saturated and cross-linked, it is very difficult to turn-over. It is reasonable to suppose that the alternation of good and poor control induces complications in any case because of the inability to remove the accumulated damages during the good control period. A study of this risk in animals is needed.

In conclusion, our data support the hypothesis that hyperglycaemia leads to collagen browning and oxidation damage, the former appearing to be faster and more intense. The data, however, suggest that the two pathways are biochemically and structurally related and have to be included in the so-called Maillard reaction. Good glycaemic control, initiated immediately after the onset of diabetes, is able to prevent the increase of both reactions.

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