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The Pecking Order of Skin Advanced Glycation Endproducts (AGEs) as Long-term Markers of Glycemic damage and Risk Factors for Micro- and Subclinical Macrovascular Disease Progression in Type 1 diabetes

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Abstract

To date more than 20 glycation products were identified, of which ~15 in the insoluble human skin collagen fraction. The goal of this review is to streamline 30 yrs of research and ask a set of important questions: in Type 1 diabetes which glycation products correlate best with 1) past mean glycemia 2) reversibility with improved glycemic control, 2) cross-sectional severity of retinopathy, nephropathy and neuropathy and 3) the future long-term risk of progression of micro- and subclinical macrovascular disease. The trio of glycemia related glycation markers furosine (FUR)/fructose-lysine (FL), glucosepane and methylglyoxal hydroimidazolone (MG-H1) emerges as extraordinarily strong predictors of existing and future microvascular disease progression risk despite adjustment for both past and prospective A1c levels. X^2 values are up to 25.1, p values generally less than 0.0001, and significance remains after adjustment for various factors such as A1c, former treatment group, log albumin excretion rate, abnormal autonomic nerve function and LDL levels at baseline. In contrast, subclinical cardiovascular progression is more weakly correlated with AGEs/glycemia with X^2 values < 5.0 and p values generally < 0.05 after all adjustments. Except for future carotid intima-media thickness, which correlates with total AGE burden (MG-H1, pentosidine, fluorophore LW-1 and decreased collagen solubility), adjusted FUR and Collagen Fluorescence (CLF) are the strongest markers for future coronary artery calcium deposition, while cardiac hypertrophy is associated with LW-1 and CLF adjusted for A1c. We conclude that a robust clinical skin biopsy AGE risk panel for microvascular disease should include at least FUR/FL, glucosepane and MG-H1, while a macrovascular disease risk panel should include at least FL/FUR, MG-H1, LW-1 and CLF.

Keywords

glycation; oxidation; retinopathy; neuropathy; nephropathy; coronary artery calcium; intima media thickness; left ventricular mass; methylglyoxal

Introduction

In 1986 we described the first association between skin collagen fluorescence as a surrogate marker of the advanced Maillard reaction in vivo and the severity of diabetic complications in Type 1 diabetes [1]. This work led to a long and fruitful collaboration with the Diabetes Control and Intervention Trial (DCCT) Group of investigators from which a large body of data resulted on the association between skin collagen AGEs and the progression of complications during the follow-up phase, named Epidemiology of Diabetes Interventions and Complications (EDIC) study. Below we summarize this work, whereby emphasis is placed on defining the “pecking order” of skin collagen AGEs for each of the associations under study during the DCCT/EDIC. The DCCT was originally designed to test the hypothesis that long-term and sustained control of glycemia to near-normal levels in Type 1 diabetes will prevent or delay the development of the microvascular complications retinopathy, neuropathy and nephropathy. A total of 1441 subjects were enrolled of which about half received intensive diabetes treatment and the other half received conventional treatment[2]. About half of the participants had no complications at baseline (primary cohort), while the other half had only background retinopathy (secondary cohort). Mean age in both groups was 27 years.

The intervention phase of the DCCT lasted on the average 6.5 years and closed in 1993. The outcome of this trial revealed that sustained lowering of HbA1c (A1c) was associated with a highly significant decrease in prevalence at all ages of all forms of microvascular complications, implying a strong role of hyperglycemia in the pathogenesis of these complications. The follow-up EDIC phase was initiated in 1994 and is still in progress. 1375 of the DCCT participants were regularly evaluated for progression of microvascular and macrovascular disease, glycemic levels and hypoglycemia. In a nutshell, as recently reviewed in several publications [3–5], the EDIC trial uncovered a “metabolic memory phenomenon” in that complication progression was overall slower in those participants who were formerly in the intensive treatment group compared to those in the former conventional group, despite mean A1c values being similar during the EDIC phase of the study.

Major findings included a 54–76 %, 40% and 60% percent lower progression rate in retinopathy, nephropathy, and clinical neuropathy, respectively. In addition, subclinical macrovascular progression indices were also muted, as revealed by a 52% reduction of CAC deposition score > 200 in the primary cohort [6] at EDIC year 8, and a 17% lower progression of carotid intima-medial thickening (cMIT) in EDIC years 1–12 [7], though no improvements in cardiac endpoints measured by MRI (ejection fraction, LV mass, LV mass/end-diastolic volume, arterial stiffness) were noted [8]. Most recently, it was found that major cardiovascular events (fatal and non-fatal myocardial infarct (MI) and stroke) which affected about 10% of the original participants were significantly lower by 42% in the former intensive treatment group [8]. Moreover, of special significance, intensive treatment was associated with a 33% reduction in all-cause mortality from baseline to a mean of 27 years of follow-up time ($p=0.045$)[8].

The conclusion of these clinical studies is that controlling hyperglycemia for extended period of time slows the rate of both micro- and macrovascular disease in Type 1 diabetes, if

started early and sustained for several years. The applicability of these findings to Type 2 diabetes appears to be strongly dependent on how soon glycemic control is initiated [8,9].

The design of the DCCT/EDIC trial and other similar trials was in part made possible through the discovery of A1c as an early glycation product and marker of mean glycemia over the preceding 1–2 months. This discovery catalyzed research into advanced glycation endproducts (AGEs) and the Maillard Reaction in vivo with a focus on long-lived proteins susceptible of accumulating AGE, such as skin collagen whose half-life is 15 years [10]. Thus, an ancillary DCCT study was initiated at DCCT closeout in 1993 using fluorescence as a surrogate marker for yet unknown AGEs, based on the earlier demonstration by some of us that such fluorescence was highly associated with severity of retinopathy, nephropathy, joint and arterial stiffness in Type 1 diabetes [1].

In several publications from our laboratory and coworkers we have shown that glycation products 1) reflect cumulative glycemia over several years, 2) were responsive to lowering of glycemia during the DCCT, and 3) are associated with severity of micro- and subclinical macrovascular disease at various time-points during EDIC [11–16]. Various statistical models were tested to find out how each of them behave as markers of risk progression upon adjustment for age, diabetes duration, mean glycemia (A1c) and selected risk factors. Eventually this resulted in a large number of data, some of which can be confusing to the clinician facing the question of which AGE should be used to determine the future risk of complications progression. Thus, the purpose of this review is to provide a synthetic, streamlined overview of the studies mentioned above with the goal of identifying those skin collagen AGEs and other modifications that are *most strongly* associated with indices of past glycemia as well as the prevalence and future risk of complication progression of Type 1 diabetes despite adjustment for A1c and other variables.

Types and significance of skin collagen AGEs and solubility markers determined in the DCCT

At DCCT closeout in 1993, two skin biopsies were obtained in 216 participants[15]. The first biopsy was processed to isolate the highly insoluble collagen fraction from which a first set of seven collagen solubility markers and then available glycation products were determined (Table 1). The second biopsy was processed almost 20 years later (2012) for the assay of novel AGEs by LC/MS mass spectrometry, eventually resulting in a total of 14 markers that are listed in Table 1. It should be noted that acid hydrolysis was used to measure the acid resistant AGEs CML, pentosidine and furosine, which is an acid stable conversion product of fructose-lysine. Furosine and fructose-lysine should provide the same information. De facto, however, we noticed some differences in the results, which we attribute to technical differences affecting the yield, presumably because of incomplete release of fructose-lysine and instability of either furosine and/or fructose-lysine during sample processing.

Below we address three questions of pragmatic interest for the purpose of choosing the best possible marker(s) for clinical correlations, and refer the reader to the original publications for more details.

1. Which skin marker in Table 1, regardless of the method used, correlates most strongly with past glycemia and the severity of microvascular disease despite adjustment for past mean A1c over several years?
2. Which skin marker correlates most strongly with severity of microvascular disease at the time of the biopsy?
3. Which skin marker has the highest potential to predict the future progression risk of micro- and macrovascular disease even after adjustment for future mean glycemia ?

The pecking order of the correlation between skin collagen AGEs and mean glycemia represented by mean A1c values over many years

Which skin AGE marker correlates most strongly with past glycemia and the current severity of microvascular disease despite adjustment for mean A1c up to the time of the biopsy?

This is the question an investigator might want to ask to understand the “total AGE burden” a diabetic individual has been exposed to in the past. This information can be important in two settings. First, anti-complication drug intervention trials might reveal that patients whose total AGE burden is high will respond poorly to pharmacological intervention due to the “memory phenomenon”[17]. At this time, there is scanty experimental data on this question. The basis of metabolic memory may lie in epigenetic changes[18] or the total AGE burden itself. It is conceivable that an intervention trial’s poor response to a given anti-diabetes therapy is related to the total AGE burden. Thus, in the emerging concept of personalized medicine, knowledge of the tissue AGE burden could prove essential to potentially avoid useless drug therapy and needless exposure of the patient to drug toxicity. Conceivably, the failed average response to intensive glycemic treatment in the ACCORD, VADT and ADVANCE trials[19] could have masked a favorable response in subjects with low tissue burden of AGEs, if the latter data had been available.

The second situation in which knowledge of the AGE burden might be useful is, for example, for assessing the risk of poor engraftment of tissue from live or dead diabetic donors, such as for corneal, bone marrow and other transplants if the deleterious aspect of the memory phenomenon persists in the host. This issue is of growing concern as the number of tissue donors with diabetes is expected to soon reach 30%.

Table 2 summarizes the hierarchical correlation between skin AGEs and mean DCCT A1c levels after adjustment for age and diabetes duration. Fructose-lysine (or furosine for that matter), the early glycation/Amadori product and precursor of glucosepane, emerges as the best marker of cumulative glycemia over many years. Indeed, while many AGEs are very significantly related to mean glycemia (mean A1c), as reflected by the p values (all of which are less than 0.0001), the R^2 values are the strongest for fructose-lysine/furosine. This is somewhat surprising, because fructose-lysine is slowly reversible and prone to oxidation, fragmentation and formation of glycoxidation and other AGE products. Thus, one would expect stable AGEs to correlate even better with mean A1c over the entire duration of the

DCCT. Nevertheless the AGE glucosepane, whose structure contains the full six carbon atoms of glucose itself, is the second highest marker of cumulative glycemia.

In contrast, those AGEs whose formation requires chemical or biological transformation of glucose into circulating AGE precursors, such as methylglyoxal and glyoxal for MG-H1, CEL or G-H1, showed the weakest or even no association with cumulative glycemia. Interestingly, pepsin-collagen solubility was strongly (inversely) related with long-term mean A1c, arguably because impaired digestibility is influenced by the extent of crosslinking by glucosepane as well as AGE-mediated blockage of protease binding and digestion sites.

From this analysis, we conclude that, in a person with Type 1 diabetes, determination of skin collagen fructose-lysine provides the best measure of mean glycemic exposure over many years, and that measurement of other AGEs is not expected to provide additional useful information concerning glycemic exposure. This relationship is shown for the first time in Fig. 1 which shows the expected linear correlation between mean DCCT A1c and skin collagen fructose-lysine in the DCCT diabetic subjects. Thus, tissue glycation and mean hemoglobin glycation correlate highly over many years. In this figure the red lines mark the upper normal values of glycation of both proteins, whereby the latter is defined as the mean plus two SDs for tissue fructose-lysine. Of practical significance is that we previously reported that the risk of retinopathy increased five-fold for individuals with both high hemoglobin (A1c > 9.0%) and collagen glycation levels above the third quartile, as determined by the furosine method [12].

The pecking order of skin collagen damage by glycation products as a function of age, presence of diabetes and response to glycemic control

One of the most important questions concerning glycation and the progression of diabetic complications is whether glycation products inflict sufficient damage to cells and tissues to result in tissue dysfunction and ultimately the pathogenesis of complications. An enormous amount of literature has accumulated offering various cellular and biochemical mechanisms by which AGEs are linked to cell and tissue dysfunction in vitro and in experimental models of diabetes. Various drugs with anti-glycation and anti-glycoxidative properties support a role for glycation in diabetic complications (see articles in this issue by Forbes and Brett), especially in rodent models, whereas trials in the human have had so far mixed success[20] [21] [22]. Yet, it is reasonable to expect that any tissue damage will be in part related to the total extent of modification of critical molecules, and especially related to the question of whether the protein damage affects critical matrix sites for cell binding, matrix turnover, matrix elasticity or certain cell surface and cytoplasmic molecules. Although our ancillary study was not designed to address the nature of the protein sites involved, comparative quantitative analysis of the mean levels of each AGE present in the DCCT biopsies should provide a strong basis for future research into the biological significance of site-specific damage by circulating reactive carbonyls of the Maillard reaction in vivo.

Figs. 2A and 2B shows linear plots depicting the age relationship of all early and advanced glycation endproducts and selected other oxidation products assayed by us in autopsy

specimens (Fig. 2A). These comprise 117 donors, 58 without diabetes, and 59 with Type 1 or Type 2 diabetes as previously published [23]. This comparative analysis shows that some AGEs and oxidative modifications accumulate with age (such as glucosepane, LW-1, CML, CEL, pentosidine, the lysine arginine crosslinks from methylglyoxal (MODIC), glyoxal (GODIC), deoxyglycosone in oxidized form (DODIC-OX), and 2-amino adipic acid), while others, such as furosine (fructose-lysine), DODIC, 6-hydroxynorleucine are in a steady state with age. These graphs are very useful for quick overview of the effects of diabetes (black symbols) on AGE accumulation as revealed by black data points above the 95% confidence interval for the normal population.

Thus, based on the mean value at 50 years for non-diabetics, the *pecking order of AGE damage in aging skin collagen* is as follows:

Total damage to collagen:	ornithine> fructose-lysine [*] > glucosepane>MG-H1>CML~ 2-AAA (2-amino adipic acid) ~LW-1 >> all others
Damage to lysine residues:	fructose-lysine > glucosepane>CML ~ LW-1> CEL>GOLD
Damage to arginine residues:	ornithine>glucosepane > MG-H1> CML> LW-1>> others
Crosslinking Lys-Arg residues:	glucosepane>> all others (MODIC~DODIC~ GODIC> DODIC-OX)

* Furosine is adjusted for 50–75% loss due to acid hydrolysis

From the above analysis, it is clear that arginine is the major target of damage eventually resulting in ornithine, most likely via spontaneous destruction of arginine AGEs [23,24].

The *pecking order of damage in Type 1 diabetes* is almost identical as in aging, except that upper levels are 2–4 fold elevated, especially for glucosepane (Table 3). However, ornithine was surprisingly not significantly increased by diabetes. Again, the dominant modification is glucosepane, followed by fructose-lysine, CML, LW-1 and others. In previous studies, we found glucosepane levels to be highly correlated (R^2) with fructose-lysine (36.8%), LW-1 (32.5%), furosine (19.4%), CML (16.1%) strongly suggesting that LW-1 and CML are glucose-derived. Interestingly, the correlation with MG-H1 was not significant [15], as expected based on the many intracellular transformations glucose is undergoing before yielding methylglyoxal.

Based on this table we find that the *reversibility of glycation* upon intensive treatment of glycemia up to 10 years during the DCCT follows the sequence fructose-Lysine (– 25%) > LW-1 (–23%) > glucosepane (–17%)> CML (–15%) as expected based on the known reversibility of fructose-lysine. For the other AGEs, the percentage of reversibility is negligible, suggesting that the turnover rate of the extracellular matrix is dictating the reversibility [10].

Omitted from the above analyses are levels of MOLD, GOLD, DOLD lysine crosslink dimers [25], and the purely oxidative modifications, such as methionine sulfoxide, itself a marker of complications. Previous reviews have addressed the problem of oxidation and the boundary between glycooxidation and lipoxidation in diabetes [26,27]. Yet, current reviews addressing the question of whether oxidation markers supersede glycation markers are urgently needed.

The pecking order of the skin AGE correlation with existing and future severity risk of microvascular and subclinical macrovascular disease after adjustment for mean A1c

In Table 3, we present the pecking order of the most robust associations between glycation products, microvascular complications present at the time of biopsy[28], and the future progression of both microvascular and subclinical macrovascular complications[11]. By robust, we mean associations that remain significant despite adjustment for age, diabetes duration, past and future A1c, in which multivariate regression analysis employing a backward selection method was used with Benjamini Hochberg correction for multiple AGEs. We strongly encourage the reader to read the original papers for detailed information.

The take home message of 25–30 years work on skin glycation products as markers of complication, is that the trio fructose-lysine/furosine, glucosepane and methylglyoxal Hydroimidazolone (MG-H1) is sufficient to assess the risk of progression of retinopathy, nephropathy and neuropathy despite all sorts of adjustments. Particularly surprising is how powerful collagen-linked fructose-lysine (measured as furosine) is in its association with all complications including coronary calcium deposition. MG-H1 stands out as a necessary marker for assessment of the risk of clinical neuropathy and carotid intima-media thickening. It's formation is thought to induce anoikis by inhibiting cell attachment to basement membranes [29]. However, tissue levels of MG-H1 are in a steady state with age, meaning that irreversible damage can only be mediated by the methylglyoxal crosslinks MOLD and MODIC which accumulate with age, or indirectly via cell-mediated matrix proliferation signaling and collagen deposition leading to intima media thickening (IMT).

While collagen fluorescence and LW-1, both with similar excitation emission maxima (348/463 nm for LW-1), and pentosidine are associated with microvascular complications, they stand out as the major markers of future subclinical macrovascular disease, especially IMT and cardiac hypertrophy. Overall, however, the analyses indicate that while glucose-derived glycation products are strongly linked with microvascular disease, but the association between skin AGEs, and perhaps glycemia too, and macrovascular disease progression risk is much more moderate.

Similarities and discrepancies with other studies of Type 1 diabetes

The above conclusions are strictly based on our own analyses of DCCT samples with microaneurysms and albumin excretion rates as endpoints for retinopathy and nephropathy, respectively. Yet, somewhat different results were obtained in the “Medalist Study” in which we analyzed skin biopsies AGEs using GC/MS in acid collagen hydrolysate from donors with at least 50 years of Type 1 diabetes [30]. In this group A1c did not correlate with complications. In contrast the combination of CEL and pentosidine was highly associated with the odds of any complication (Fig. 4), but furosine surprisingly did not correlate. Moreover, individuals with high levels of both furosine and CML had lower rate of proliferative retinopathy, a complication not assessed in the DCCT skin study. The reasons for these discrepancies are not entirely clear, but may relate to the older age of the Medalist

participants and the very long duration of diabetes, but perhaps also to technical problems with AGE analysis by GC/MS instead of LC/MS.

We have not reviewed here similar studies by Baynes, Lyons, Beisswenger and colleagues[31,25,32,33], mostly because they did not include the full set of AGEs presented above. However, these have been discussed in the original publications that we cited above. It should be noted here that other modifications have been recently reported by Glomb, Gillery and their respective colleagues, some of which are present or expected to be found in human collagen, such as homocitrulline[34,35].

To our knowledge the DCCT/EDIC ancillary skin AGE study described above is the largest of its kind carried out to date. However, in a recent collaboration with the investigators of the landmark Oslo Type 1 Diabetes Study[36], which asked similar questions as the DCCT but with a very small cohort, we found an association between left ventricular dysfunction with serum MG-H1 and skin collagen G-H1 in 20 type 1 diabetes patients without significant stenosis on coronary angiography when compared with 26 controls[37]. Skin glucosepane was also found to correlate significantly with cIMT ($r=0.41$) and PWV ($r=0.44$) [38], echoing some of the findings in the EDIC trial[11].

Finally, the studies above have been presented under the hypothetical applicability of skin biopsies for the clinical purpose of estimating disease risk progression. However, unless major progress is achieved with the non-invasive measurement of collagen-bound AGEs, clinicians will have to rely on the non-invasive determination of skin autofluorescence as surrogate markers for AGEs by instruments such as the AGE Reader (Diagnoptics) or the SCOUT instrument (Veralight/Miraculin), respectively. The utility of skin autofluorescence in the clinical setting is reviewed in this issue by Fokkens & Smit (REF), and the relationship between autofluorescence, skin collagen AGEs and diabetic complication progression has been recently reviewed in the context of our studies on the collagen fluorophore LW-1 [16].

Conclusions

The take home message from the above analyses is quite straight forward. Skin collagen fructose-lysine is not only the best marker of antecedent glycemia over many years, but fructose-lysine (furosine) also stands out as a major risk factor of microvascular disease. We therefore strongly recommend that it be included in all future microvascular risk panels along with glucosepane and MG-H1. The latter is important as a neuropathy marker. For macrovascular disease collagen fluorescence, fluorophore LW-1, pentosidine and MG-H1 are predictors despite adjustments for A1c and should be part of a risk panel, whereby progress in structure elucidation of skin associated fluorescence is greatly needed. In addition, there is a need to combine AGE markers with additional, more robust, long-term risk markers which have added value for risk reclassification on top of the conventionally used predictors. From a practical viewpoint, however, as long as non-invasive technology will be unavailable for collagen-linked AGEs, especially fructose-lysine, skin autofluorescence is the desired technology.

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Abbreviations

2-AAA	2-amino adipic acid
AGE	Advanced glycation endproducts
A1c	Hemoglobin A1c
FL	Fructose-lysine
FUR	Furosine
CLF	Collagen-linked fluorescence
CEL	Carboxyethyl-lysine
CML	Carboxymethyl-lysine
cMIT	Carotid intima-medial thickening
CVD	Cardiovascular disease
DCCT	Diabetes Control and Complications Trial
DODIC	Deoxyglucosone-derived imidazoline crosslink
EDIC	Epidemiology of Diabetes Interventions and Complications
G-H1	Glyoxal hydroimidazolone 1
GODIC	Glyoxal-derived imidazoline crosslink
GOLD	Glyoxal lysine dimer
IMT	Intima media thickness
LC/MS/MS	liquid chromatography mass spectrometry
LW-1	Long-wave fluorophore 1
MI	Myocardial infarct
MG-H1	Methylglyoxal hydroimidazolone
MODIC	Methylglyoxal-derived imidazoline crosslink
MOLD	Methylglyoxal lysine dimer
PWV	Pulse wave velocity

SAF	Skin autofluorescence
SIF	Skin intrinsic fluorescence
SD	Standard deviation

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Fig. 1.

Relationship between tissue collagen glycation (fructose-lysine) and mean hemoglobin glycation (A_{1c}) over several years during the DCCT. The red line at A_{1c} 6.5% corresponds to the recommended guidelines for the definition of diabetes. The red line at 5,500 pmol/mg collagen fructose-lysine corresponds to the mean value (3,675 pmol/mg) plus two standard deviations (SD = 899 pmol/mg protein) of values in the control population. Any value above this level is to be considered abnormal.

Fig. 2A

Fig 2.

Fig 2A: Linear plots showing the age-related accumulation of glycation, advanced glycation and selected oxidation product in the insoluble skin collagen fraction from autopsy donors. Sample used for these correlations were obtained at autopsy (n =117) of whom 58 had diabetes. The 95% confidence interval for non-diabetic controls is shown. 2B: Similar plots for the AGEs MG-H1, LW-1 and G-H1 generated using the data from the DCCT trial.

Fig 3.

The pecking order of skin AGEs in skin from individuals with Type 1 diabetes, with and without intensive vs. conventional control of glycemia. Data are from the DCCT trial.

Fig 4. Relative odds of complications associated with high vs. low CEL and pentosidine levels in the Joslin Medalist study [30]. Cx, complication; Pent, pentosidine. **B:** Risk of PDR development by CEL and pentosidine levels. DM, diabetes; Pent, pentosidine. (Reproduced with permission from ref [30].)

Table 1

Collagen glycation and solubility modifications determined in two skin biopsies obtained simultaneously at DCCCT closeout in 1993 but processed 20 years apart. The first seven analytes were part of the first biopsy which used acid hydrolysis to release acid stable AGEs. The second biopsy (bottom seven analytes) was processed according to the method of Thornalley et al[39].

Collagen Glycation and Solubility Marker	Abbreviation	Hydrolysis Method	Instrument	Proposed Biochemical significance/origin
Furosine	FUR	Acid	HPLC	Early glycation (Amadori)
Carboxymethyl-lysine	CML	Acid	HPLC	Glycoxidation/lipoxidation
Pentosidine	PENT	Acid	HPLC	Glycoxidation
Fluorescence at 335/365 nm		Enzymatic	Fluorimeter	Pentosidine-like
Fluorescence at 370/440nm	CLF	Enzymatic	Fluorimeter	Generic AGE marker, overlaps with LW-1 and skin autofluorescence
Collagen acid solubility		Enzymatic	OH-proline	Generic marker of crosslinking
Pepsin insoluble collagen		Enzymatic	OH-proline	Generic marker of crosslinking
Fructose-lysine	FL	Enzymatic	LC/MS	Early glycation (Amadori)
Carboxyethyl-lysine	CEL	Enzymatic	LC/MS	Mixed origin: methylglyoxal, ascorbate,
Methylglyoxal hydroimidazolone 1	MG-H1	Enzymatic	LC/MS	Methylglyoxal
Glyoxal hydroimidazolone 1	G-H1	Enzymatic	LC/MS	Glyoxal (from lipids, ascorbate, glycoxidation)
Glucosepane	GSPN	Enzymatic	LC/MS	The major glucose-derived AGE and crosslink
Long-Wave fluorophore 1	LW-1	Enzymatic	LC/MS	A major lysine AGE of unknown origin
Methionine sulfoxide	MSOX	Enzymatic	LC/MS	A marker of oxidative stress

Table 2

The pecking order of age- and diabetes-duration adjusted skin collagen AGEs with mean glycemia over 6–10 years in Type 1 diabetes. This table was created using data from references [13] and [28].

Collagen Modification	R ² %	P-Value
Fructose-lysine/Furosine	36.8 /19.4	<0.0001
Glucosepane	23.5	<0.0001
CML	16.1	<0.0001
Pepsin-soluble collagen	15.0	<0.0001
LW-1	10.1	<0.0001
Pentosidine	8.9	<0.0001
Fluorescence (370/440nm)	6.5	<0.0001
MG-H1	0.4	NS
CEL	0.2	NS
G-H1	0.1	NS

Table 3

The pecking order of the most robust skin collagen glycation markers that still remain significantly associated with existing complications and future long-term progression risk of at least one complication event after multivariate regression analysis or backward selection in spite of adjustment for age, diabetes duration, A1c, or other selected factors¹

Complication	Marker	Association with complications existing at the time of biopsy ²			Association with 13–16 year future complication progression (adjusted for EDIC mean A1C)		
		R ² %	p value	Ref	X ²	p value	Ref
Retinopathy	FUR				25.1	<0.0001	11, Table 3
	GSPNE	5.8/15.1	0.003	28	24.2	<0.0001	
	FUR/GSPNE ³	3.6	0.025	28	14.5	0.0006	
Nephropathy	FL	7.4	0.005	28	13.1	0.0003	11, Table 4
	GSPN	11.0	0.001	28	11.7	0.0006	
	FUR adj for AGEs ³ for sel.factors ³				3.9	<0.05	
	G-H1, MG-H1, GSPN	30.3	<0.0001	28			
Neuropathy	FUR				7.8	0.005	11, Table 5
	GSPNE	9.3	0.015	28			
	MG-H1				16.1	<0.0001	
	FUR+MG-H1 ³				18.5	<0.0001	
Coronary artery calcium score	FUR				4.4	<0.05 ⁴	14
	Fluorescence					<0.05	16
Intima-Media Thickness	MG-H1				2.7 (t value)	0.008	14
	Pentosidine	Not enough events for these correlations			2.3 (t value)	0.022	14
	LW-1					<0.05	16
Cardiac MRI studies	LW-1					<0.05	16
	Fluorescence					<0.05	

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¹For details, refer to the original publications

²From Table 4 and 5 ref. 28: analysis limited to those with no respective complication at DCCT baseline. Refer to original reference for details

³Backward adjustment for selected AGEs remaining significant after adjustment for various variables such as AGEs, A1c, retinopathy and log (AER) at EDIC baseline

⁴After adjustment for EDIC A1c but not DCCT A1c