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Research Article

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Glucosylation of Human Collagen in Aging and Diabetes Mellitus

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ABSTRACT Several of the characteristic complications of diabetes mellitus resemble age-like changes in collagen-rich tissues. It has been reported that increased glucosylation of hemoglobin and serum proteins occurs in diabetes. Glucosylation of insoluble human tendon collagen, a protein with little or no turnover, was determined by a thiobarbituric acid method in 23 subjects as a function of age and the presence or absence of diabetes. Amounts of glucose and collagen solubilized by collagenase digestion of the samples were also determined. Glucosylation of collagen was found to increase with age and was markedly increased in juvenile onset diabetes. There appeared to be a limit to the amount of glucosylation that could occur, and older individuals with maturity-onset diabetes demonstrated glucosylation within that limit. The glucose nonenzymatically bound to human collagen may indicate the level of long-term control of the diabetes, and may play a role in the alteration of collagenous tissue properties that occurs in both aging and diabetes.

INTRODUCTION

Glucose has been shown to bind nonenzymatically to a variety of proteins through Schiff base formation, and amadori rearrangement to a ketoamine derivative (1–3). Glucosylation appears to be a function of both the duration of exposure and the concentration of glucose (4, 5).

Previous reports have described the glucosylation of proteins with relatively high turnover rates, and an increased glucosylation of proteins from patients with diabetes mellitus (1, 3, 6).

We measured the glucosylation of insoluble human tendon collagen, a substance with little or no turnover (7), as a function of age and in several subjects with diabetes mellitus.

METHODS

Human diaphragmatic tendon samples were obtained at autopsy and insoluble collagen was prepared as previously described (8). 16 samples from individuals without diabetes or any disease involving connective tissue were studied, along with samples from two individuals with long-standing juvenile-onset diabetes (JOD),[1] and five samples from individuals with well-documented maturity-onset diabetes (MOD). The amount of collagen was determined by measurement of hydroxyproline (Hyp), assuming that Hyp represents 14% of the weight of collagen (8). Collagen comprises over 99% of the organic matter of the samples (8). Samples of lyophilized insoluble material were suspended in distilled water and homogenized in a glass tissue grinder. Final concentrations of suspensions were 4–6 mg/ml.

Glucosylation of tissue was measured by a modification of the thiobarbituric acid method of McFarland et al. (3). After incubating suspensions in 1 M oxalic acid for 4.5 h, the samples were centrifuged at 300 *g* for 15 min and filtered through Whatman No. 42 filter paper (Whatman, Inc., Clifton, N. J.) 1-ml aliquots were removed for measurement of 5-hydroxymethylfurfural (5-HMF). Glycosidically bound glucose can only be degraded to its furfural derivative with strong mineral acids (9); such glucose would not be determined by this procedure.

Samples of insoluble collagen were digested with collagenase (EC 3.4.24.3, Worthington Biochemical Corp., Freehold, N. J.) under conditions previously described (8). Aliquots of the digestion mixture were removed at 1 and 24 h and centrifuged at 29,000 *g* for 30 min. The undigested residues were washed three times and assayed for Hyp (8) and 5-HMF (3).

RESULTS

The amount of glucose released from insoluble collagenous tissue as 5-HMF, increases with age, as shown in Fig. 1 ($r = 0.903, P < 0.001$). Two JOD and one MOD have significantly more glucose bound to their collagenous tissue via the ketoamine linkage than would be expected for their ages ($P < 0.01$). The older MOD, however, have values within the range expected for nondiabetics of the same age.

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[1] *Abbreviations used in this paper*: 5-HMF, 5-hydroxymethylfurfural; Hyp, hydroxyproline; JOD, juvenile-onset diabetes; MOD, maturity-onset diabetes.

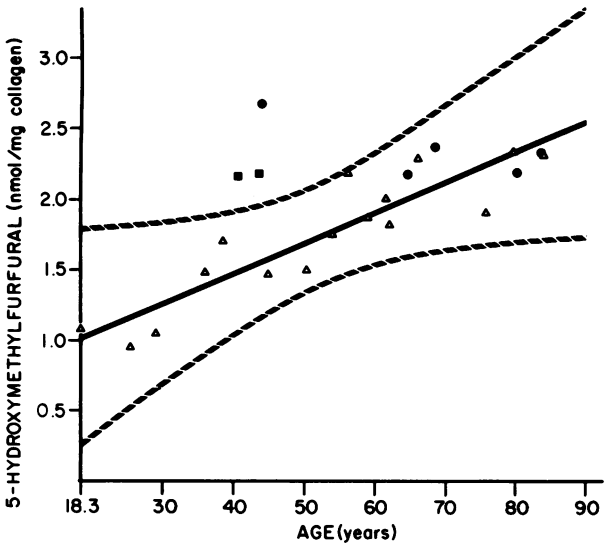

FIGURE 1 The concentration of ketoamine-linked, collagen-associated hexose as measured by the amount of 5-HMF released per milligram of collagen, is plotted as a function of subject's age. The solid line represents the regression equation: $y = 0.043(\times) + 1.25, r = 0.903, P < 0.001$ and is derived from the data of nondiabetics. The 99% confidence bands are represented by the dashed lines. Nondiabetic subjects (Δ, $n = 16$); JOD subjects (■, $n = 2$) and MOD subjects (●, $n = 5$).

The 1-h digestion data in Table I are consistent with previous reports that insoluble collagen becomes resistant to collagenase digestion with increasing age (8, 10). The fraction of original ketoamine-linked glucose remaining in the residue after digestion did not show any consistent age associated differences. However, more ketoamine-linked glucose was released with the collagen from old compared to mature subjects after both 1 and 24 h of digestion. Although 97.0–99.6% of the collagen was digested after 24 h, significant fractions of the starting ketoamine-linked glucose remained in the undigested residue. However, as indicated in Table I, between 72.1 and 87.5% of the measured ketoamine-linked glucose is associated with collagen and this collagen-linked glucose increases with age.

DISCUSSION

Insoluble human collagen has been shown to undergo a number of changes as a function of age after maturity, including resistance to collagenase digestion (8). Resistance to collagenase was also found to be very marked in collagen from individuals with diabetes mellitus (10). Our data indicate that there is also an increase in glucosylation of collagen with age and diabetes. A reducible lysine-carbohydrate condensation product from tendon collagen that increases with age has been reported (12). Another study, using the same technique, concluded that this reducible fraction increased with age up to 38 yr and then decreased to approximately the level measured in a 2-yr old (13). It is not known if the increased glucosylated collagen with age we report here is the same as the reducible fraction described in these reports.

It should be noted that the curve in Fig. 1 applies to insoluble collagen present during the postmature period. Such collagen comprises over 99% of the total collagen present in human tendon (8). This collagen is also relatively inert. During growth, there is extensive collagen synthesis, much of the collagen is soluble, and this collagen undergoes significant turnover (7). It would therefore not be justified to extrapolate the curve in Fig. 1 back through the period of growth and development.

It is interesting that older MOD did not have more glucosylated collagen than their age matched controls. A possible explanation for this is that in postmature insoluble tendon collagen there is a limited number of sites available for glucosylation and that these sites are occupied early during the course of hyperglycemia. In support of this hypothesis, the amount of ketoamine-linked glucose released per microgram digested collagen was the same in the 61.9-yr-old sub-

TABLE I
Effect of Collagenase Digestion on Measurement of Ketoamine-linked Glucose in Samples of Insoluble Human Collagen

	1-H digestion			24-H digestion		
Subject age	Collagen remaining*	5-HMF remaining	5-HMF released/ Hyp released	Collagen remaining*	5-HMF remaining	5-HMF released/ Hyp released
yr	%	%	*ng/μg*	%	%	*ng/μg*
18.3	2.20	36.50	0.66	0.40	12.50	0.89
25.5	0.68	44.50	0.51	0.49	17.30	0.76
61.9	25.00	45.60	1.32	3.00	27.90	1.35
75.7	19.80	42.70	1.14	0.90	16.10	1.35

* Maximum possible collagen, if all the Hyp remaining were in collagen.

ject and the 75.7-yr-old subject, even though the 61.9-yr-old subject had greater amounts of both Hyp and ketoamine-linked glucose remaining after collagenase digestion. Although the number of sites available for glucosylation must be limited, there is no direct evidence that they are saturated in the samples studied. This question is amenable to investigation.

Our demonstration of an increased glucosylation of collagen in the JOD and the young MOD subject is in agreement with a recent report by Rosenberg et al. (14) in which streptozotocin induced diabetes in rats was found to result in an increased glucosylation of aortic collagenous material. The purity of their collagen preparation, however, was not documented.

It is possible that increased glucosylation of collagen occurring with age and in the presence of diabetes, plays a role in the age-like complications of diabetes that occur in collagen-rich tissues (15–17).

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