Acta Paediatrica Academiae Scientiarum Hungaricae, Vol. 23 (1), pp. 27—34 (1982)

Glycosaminoglycan and hydroxyproline excretion in diabetic children

L. Klujber, Rozalia Szücs, Zsuzsa Baranyai, J. Gabulya, Gy. Soltész

Department of Paediatrics, University Medical School, Pécs

Glycosaminoglycan and hydroxyproline excretion was studied in 24 diabetic children. While diabetic children under satisfactory control had a normal excretion rate, hyperglycaemic, ketotic patients excreted more glycosaminoglycan and hydroxyproline than did the controls. The high urinary glycosaminoglycan excretion was found to be due to increased excretion of hyaluronic acid.

Metabolism of collagen, the major connective tissue protein, is affected in diabetes [2, 5]. According to recent data [11, 12] the excretion rate of the metabolic end product of the macromolecules is also altered. In addition, the excretion rate of hydroxyproline (HYP) and glycosaminoglycans (GAG) may also be influenced by growth retardation which is often associated with diabetes [7].

Material and Methods

Subjects. Twenty four children suffering from insulin-dependent diabetes mellitus (IDDM) and 62 healthy, age and sex matched children were studied. On the basis of the presence of ketone bodies in the urine sample at the day of examination, patients were divided into ketotic and non-ketotic group. The reason of hospitalization of the ketotic children was either the first admission for the diagnosis of diabetes mellitus (6 cases) or infections, diet-breaking resulting in hyperglycaemic ketosis. The non-ketotic children were under satisfactory control. Relevant data of the two groups are summarized in Table I.

Laboratory methods. Apart from routine sampling, 24 hours urine samples were collected and stored at —20 °C. Total and free HYP were determined according to Kivirikko et al. [8]. Macromolecular GAG was isolated by cetylpyridinium chloride (CPC). Fraction was done by cellulose-Celite column chromatography [13]. Excretion of total GAG was calculated as sum of GAG fractions and expressed as equivalent of uronic acid. Fragment GAG was isolated from the supernatant of CPC isolate on ion-exchange column [4]. Uronic acid was determined with borax-carbasole and glucose with the anthrone reaction.

Serum glucose was measured enzymatically (GOD-POD Galenofarm) and urinary sugar by the o-toluidine method. Ketone bodies were detected by Acetest (Ames). HbA_{1c} and serum glycosyl protein were measured by a modified colorimetric method [10].

For statistical analysis, Student's t test and linear regression analysis were used.

This work is dedicated to Professor Julius Mestyán on the occasion of his 60th birthday.

Supported by the Scientific Research Council, Hungarian Ministry of Health (3—23—1101—02—01 V and 3—23—0701—03—1/V).

TABLE I

Characteristics of the study groups

	No. of children	Age, years	Duration of the disease	Daily blood glucose, mean ± S. D. mmol/l	Urinary glucose, mmol/day	Urinary ketone	HbA_{1c}	Serum glycosylprotein, μmol/g protein
Diabetic, non-ketotic	17	10.2 3–16	3.1	10.11±2.91	604±428	—	4.6	1.43
Diabetic, ketotic	22	11.3 3–16	2.8	16.10±3.89	1908±1194	+ − ++++	5.7	1.70
Control	62	10.9 3–16	—	4.20±0.90	—	—	2.8	0.96

RESULTS

Hydroxyproline excretion

Individual hydroxyproline excretion rates are shown in relation to the normal range obtained in healthy children (Fig. 1). In the diabetic children high values were mainly found. There was no difference between sexes as well as between newly diagnosed cases and those under long-term treatment.

The diabetic and normal children were divided into three age groups in order to see the age-related changes in hydroxyproline excretion. From Table II it is evident that both in diabetic and normal children hydroxyproline excretion increases with age. Though the mean HYP excretion rate in the three age groups of diabetic children showed a somewhat higher level than in the controls, the difference was not found to be significant.

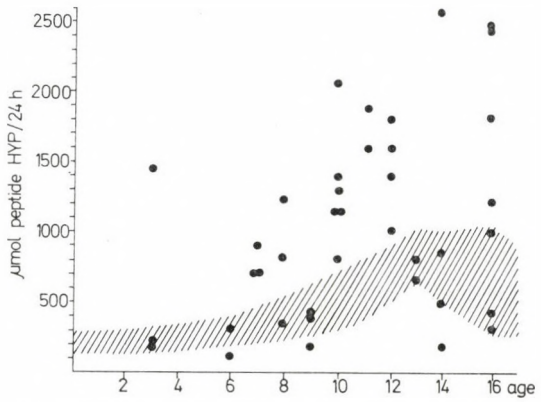

FIG. 1. Peptide hydroxyproline excretion. Normal range and individual values of diabetic children

TABLE II

Urinary HYP excretion in different age groups of diabetic and healthy children, (μ mol/24 h $\pm$ S. D.)

	Age groups		
	< 8 years	8–11 years	11–16 years
Diabetics	621.4$\pm$451.1 n = 12	665.0$\pm$497.3 n = 14	850.5$\pm$817.2 n = 21
Controls	270.1$\pm$98.0	560.1$\pm$110.3	720.2$\pm$396.3

TABLE III

Hydroxyproline excretion in normal, non-ketotic, and ketotic diabetic children

	Peptide HYP, μmol/24 h	Free HYP, μmol/24 h	Free HYP, per cent of total
Diabetic, non-ketotic	545.8$\pm$340.0	65.8$\pm$42.0	12.1
Diabetic, ketotic	1425.3$\pm$640.7	63.7$\pm$39.4	4.5
Normal	662.6$\pm$188.5	13.9$\pm$9.3	2.1

TABLE IV

Urinary GAG excretion in different age groups of diabetic and healthy children (μmol uronic acid/24 h $\pm$ S. D.)

	Age groups		
	< 8 years	8–11 years	11–16 years
Diabetics	33.4$\pm$27.5 n = 12	26.2$\pm$15.6 n = 14	25.4$\pm$18.4 n = 21
Controls	13.7$\pm$6.2	19.2$\pm$10.7	22.4$\pm$11.1

TABLE V

Glycosaminoglycan excretion in diabetic and normal children (mean, expressed as μmol uronic acid/day $\pm$ S. D.)

	Total GAG	Fragment GAG	Macromolecular/fragment GAGs	Hyaluronic acid	Chondroitin sulfate	Dermatan sulfate
Diabetic, non-ketotic	14.3$\pm$5.2	22.3$\pm$17.7	0.64	2.6$\pm$0.4	7.8$\pm$2.5	0.7$\pm$0.5
Diabetic, ketotic	44.9$\pm$20.4	43.3$\pm$24.7	1.03	25.2$\pm$15.9	13.3$\pm$4.6	1.4$\pm$1.3
Control	20.1$\pm$6.1	41.9$\pm$23.6	0.48	1.6$\pm$0.6	12.7$\pm$4.1	0.4$\pm$0.3

Evaluation according to the degree of metabolic control is shown in Table III. Peptide HYP excretion of ketotic, diabetic children was higher than either that of normal controls ($p < 0.01$) or that of diabetic but non-ketotic patients ($p < 0.01$). HYP excretion in non-ketotic children corresponded to the level of normal children.

As to free HYP in diabetic children, it was significantly higher than in the normal group. Free HYP excretion in non-ketotic diabetic children amounted to 12.1%.

Glycosaminoglycan excretion

The rate of total macromolecular GAG excretion showed a tendency similar to that of HYP (Fig. 2). GAG excretion rate seemed to be independent of sex and the duration of diabetes.

Mean excretion in different age groups is summarized in Table IV. Diabetic children excreted more GAG than controls but only in the youngest age group was the difference significant statistically.

Table V. summarizes the results according to the degree of metabolic control.

In the children with satisfactory metabolic control (non-ketotic group), GAG excretion was lower than in the controls but in the ketotic hyperglycaemic children it was significantly higher than in healthy ($p < < 0.001$) as well as in non-ketotic diabetic children ($p < 0.01$).

Excretion rates of CPC non-precipitable GAG (fragment GAGs) were the same in the three study groups. Mean values of the excreted quantities of fragment GAGs and their relation to the macromolecular GAG are given in Table V.

Individual GAG fractions showed characteristic patterns in the three groups (Fig. 3). The large amounts of GAG excreted by ketotic children differed substantially in their compo-

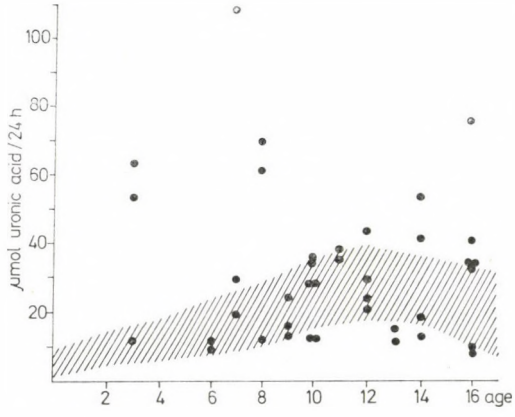

FIG. 2. Macromolecular glycosaminoglycan excretion. Normal range and individual values of diabetic children

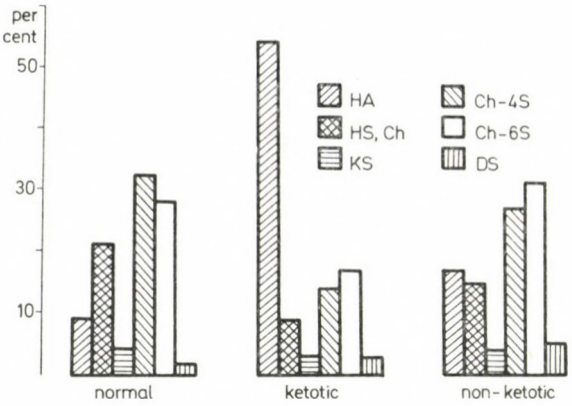

FIG. 3. Quantitative distribution of GAG components in the urine of normal and diabetic children. HA = hyaluronic acid, HS, Ch = heparan sulfate, chondroitin, KS = keratan sulfate, Ch-4S = chondroitin 4-sulfate, Ch-6S = chondroitin 6-sulfate, DS = dermatan sulfate

sition from that excreted by healthy or non-ketotic children. While in normal children, 30% of urinary GAG excretion was chondroitin sulfate, in the ketotic diabetics, hyaluronic acid constituted more than 50% of the urinary GAGs (range, 38.8% to 77.4%).

Due to the method applied, the "hyaluronic acid" fraction of the urinary GAGs is chemically heterogeneous. Besides hyaluronic acid it contains non-sulfated, low sulfated and low molecular weight GAG metabolites. There was no difference between the diabetic and control groups in the excretion rate of keratan sulfate, heparan sulfate and chondroitin. Though the ketotic group excreted three times more dermatan sulfate than did the other two, the difference was not significant statistically.

The quantity of urinary chondroitin sulfates was found to be the same in ketotic and control children. The somewhat lower excretion rate of chondroitin sulfates in the non-ketotic group turned out to be significant.

The hyaluronic acid fraction of GAGs was ten times higher in ketotic than in non-ketotic and control children ($p < 0.001$). The high GAG excretion in diabetes is mainly the consequence of the altered hyaluronic acid excretion.

Correlation between urinary connective tissue metabolites and other biochemical parameters in diabetes

A close correlation was observed between peptide HYP and total GAG excretion in diabetic children ($p < 0.01$). Furthermore, a significant correlation was found between

a) HYP excretion and the mean daily blood glucose values;

b) GAG excretion and the mean daily blood glucose values;

c) urinary HYP and daily sugar excretion (Fig. 4). On the other hand, there was no correlation between urinary metabolites (HYP, GAG) and HbA_{1c}, glycosylated serum protein levels, the daily insulin dose, and urinary glucose.

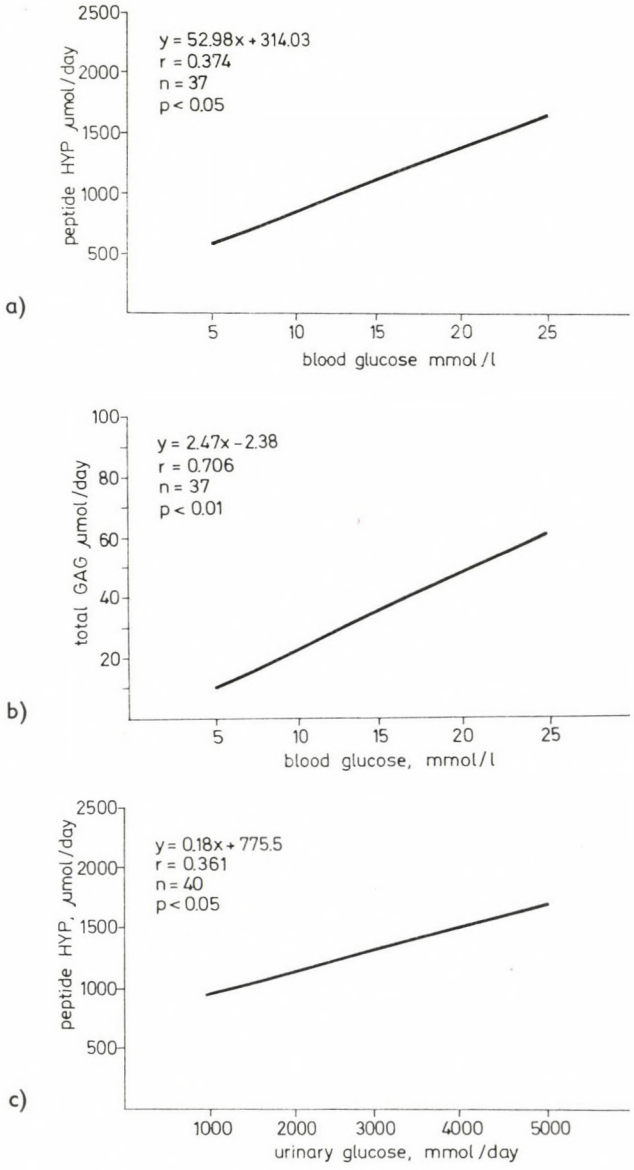

FIG. 4. Correlation between: *a)* Urinary peptide hydroxyproline excretion and mean blood glucose level. *b)* Urinary total glycosaminoglycan excretion and mean blood glucose level. *c)* Urinary peptide hydroxyproline and urinary sugar excretion

Discussion

Abnormalities in the metabolism of connective tissue macromolecules and the excretion rate of the metabolites in diabetes mellitus have been reported [1, 3, 6, 7, 11]. In animals with experimentally induced diabetes, the GAG content decreased and the soluble collagen increased [2]. Some authors, however, observed a higher hyaluronic acid content in "diabetic" than in normal cell line [5]. Total GAG excretion rate and the GAG/creatinine ratio were found to be higher in diabetic patients than in controls [6, 11]. Bodadonna et al reported decreased HYP excretion in diabetics [3], but other authors found it to be normal [1, 8] while o-hydroxylysyl glycosides, another characteristic collagenous metabolites present in urine, were excreted at lower rates than in controls [12].

On the basis of the present findings it has been concluded that in diabetic patients connective tissue metabolism and the excretion of different metabolites depended on the actual clinical and biochemical status. A diabetic child under good metabolic control grows normally, with undisturbed connective tissue metabolism and normal HYP and GAG excretion rates. During a one week period, patients may excrete HYP and GAG at high and normal rates, as we have frequently observed.

Connective tissue metabolism may rapidly change when HYP and GAG excretion correlates well with the rapidly changing parameters like blood glucose and urinary glucose excretion. In well-controlled out-patients, Lubec et al observed a correlation between GAG excretion and HbA_{1c} level [11].

Our results are in accordance with that obtained with cell lines of diabetic animals. These cultured cells not only contained but also excreted more hyaluronic acid than did normal cells [5]. It was interesting to note such a high hyaluronic acid level in the urine of intrauterine malnourished infants; in all probability it must have been due to the increased growth rate often seen in such neonates after birth [9].

The present results suggest that in diabetes the rate of connective tissue metabolism is altered and this is reflected in simultaneous changes in both HYP and GAG excretion. The high free HYP excretion in diabetic children is presumably caused not only by changes in collagen degradation, but by other metabolic alterations and changes in the renal threshold of this amino acid.

References

1. Benoit FL, Threil GB, Watten RH: Hydroxyproline excretion in endocrine disease. Metabolism 12:1072, 1963
2. Berenson GS, Radhakrishnamurthy B, Dalferes ER, Ruiz H, Srinivasan SR, Plavidal F, Brickman F: Connective tissue macromolecular changes in rats with experimentally induced diabetes and hyperinsulinism. Diabetes 21:733 1972
3. Bodadonna G, Sonenberg M, Merlino MJ: Total urinary hydroxyproline excretion in diabetics before and after hypophysectomy and after growth hormone in adults hypopituitorism. Metabolism 14:832, 1965

4. DiFerrante NM, Neri G, Neri ME, Gogsett WE: Measurement of urinary glycosaminoglycans with quarternary ammonium salts: An extension of the method. Connect Tissue Res 1:93, 1972
5. Ginsberg LC, Wyse BM, Chang A: Analysis of glycosaminoglycan from diabetic and normal Chinese hamster cells. Diabetes 30:393, 1981
6. Kennedy JF: Chemical and biochemical aspects of the glycosaminoglycans and proteoglycans in health and disease. In: Advances in clinical chemistry. ed. Bodansky O, Latner AL. Academic Press, New York, San Francisco, London 1976. P 1
7. Kivirikko KI, Laintinen O: Clinical significance of urinary hydroxyproline determinations in children. Ann Paediatr Fenn 11:148, 1965
8. Kivirikko KI, Laintinen O, Prockop DJ: Modifications of a specific assay for hydroxyproline in urine. Anal Biochem 19:249, 1967
9. Klujber L, Sulyok E: Urinary glycosaminoglycan excretion in normally grown and growth retarded neonates II. Quantitative distribution of glycosaminoglycans. Acta Paediatr Acad Sci Hung 14:209, 1973
10. Klujber L, Baranyai Zs, Gabulya J: An optimalized micro-method for colorimetric determination of glycosylated proteins. J Clin Chem Clin Biochem 19:733, 1981
11. Lubec G, Legenstein E, Pellak A, Meznik E: Glomerular basement membrane changes, HbA_{1c} and urinary excretion of acid glycosaminoglycans in children with diabetes mellitus. Clin Chim Acta 103:45, 1980
12. Sato T, Saito T, Kokubun M, Ito M, Inoue M, Saito K, Yoshinaga K: Urinary excretion of o-hydroxylysylglycosides in diabetes mellitus. Tohoku J Exp Med 131:97 1980
13. Tanaka Y, Gore I: Cellulose column chromatography for the fractionation and isolation of acid mucopolysaccharides. J Chromatogr 23:254, 1966

Received October 11, 1981

L. KLUJBER, PhD
József A. u. 7.
H-7623 Pécs, Hungary