Clinical Utility of Nonenzymatically Glycosylated Blood Proteins as an Index of Glucose Control

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This study compares the utility of nonenzymatically glycosylated serum proteins (lys-GSP) to glycosylated hemoglobins (HbA_{1a-c}) as control indices of glucose homeostasis in patients with IDDM. The diagnostic value of lys-GSP was also examined in patients with non-insulin-dependent diabetes mellitus, in subjects with impaired glucose tolerance, and in two patients with insulinoma. The intraindividual fluctuation of lys-GSP in normoglycemic subjects is very small, resulting in an interindividual range of 3.0 ± 0.3 lysine-bound glucose/mg protein ($\bar{x} \pm$ SD, N = 52). HbA_{1a-c} with a normal range of $6.4 \pm 0.9\%$ (N = 52) shows greater variability. In IDDM there is no overlap of lys-GSP levels between the normal and the diabetic range at the 95% confidence level. In patients treated with an open-loop insulin delivery system failure of normalization of the glucose balance was clearly discernible by an elevation of GSP. In contrast, in about 40% of the patients with incomplete glycemic control the HbA_{1a-c} levels fell within the normal range. The utility of lys-GSP for diagnosis of diabetes is compared with the results of 60 oral glucose tolerance tests. Two patients suffering from insulinoma displayed decreased lys-GSP values. From these results it appears that determination of lys-GSP represents a more sensitive parameter for long-term control than HbA_{1a-c} and is suitable for monitoring even small fluctuations of blood glucose. DIABETES CARE 1984; 7:548–56.

Nonenzymatic glycosylation is a general phenomenon occurring with most, if not all, proteins in vivo. Glycosylated hemoglobins (Hb) are widely used for assessment of long-term diabetes control, but it appears that other blood proteins might also represent a valuable tool in assessing diabetes control.[1–8] A variety of methods has been described for the determination of glycosylated proteins, especially for the glycosylated hemoglobins, but many of them have serious pitfalls.[9] From our experience the macrocolumn method of Schneck and Schroeder[10] as modified by Trivelli et al.[11] is the most reliable method for HbA_{1a-c} determination, although it is too laborious for routine work. The thiobarbituric acid method, performed under strictly standardized conditions,[12] also yields valid results.

For determination of glycosylated serum proteins (lys-GSP), the furosine method, recently developed in our laboratory,[7] has many advantages. Lys-GSP indicates that glucose is bound to lysine residues of the serum proteins. Here we present a comparison of the latter two methods with respect to their discriminative power. For that purpose lys-GSP were measured in normal subjects, patients with impaired glucose tolerance, diabetic patients under different therapeutical regimens, and two insulinoma patients.

METHODS

Subjects. Four groups of subjects participated in this study. The first group (group A) consisted of healthy normal subjects, 27 women and 25 men aged 20–56 yr. The second group (group B) contained 72 patients, 34 women and 38 men aged 12–85 yr, with insulin-dependent (IDDM) (N = 49) or non-insulin-dependent diabetes mellitus (NIDDM) (N = 23) diagnosed according to the criteria of the National Diabetes Data Group.[13] Heparinized blood and serum were obtained from fasting subjects of both groups for determination of glycosylated hemoglobins and glycosylated serum proteins, respectively.

Twenty sera, each randomly selected from groups A and B, were further fractionated electrophoretically[14] to deter-

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TABLE 1
Clinical characteristics of the 10 diabetic patients (group C) of the longitudinal study

Patient	Sex	Age (yr)	Duration of diabetes (yr)	Duration of follow-up (mo)	Mode of insulin treatment	Diabetic late complications
SR	F	31	2.5	10	CSII	None
NO	M	45	38	14	CSII	PR,N,Ne,M
MH	F	30	27	11	CSII	PR,Ne,N
SB	M	31	4.4	11	CSII	Ne
ZK	F	22	7	13	IVII	BR,Ne
DR	F	43	17	18	IVII/CSII	BR,Ne
FH	F	19	4	5	CSII	None
HP	M	44	0.5	6	CSII	None
SI	F	39	6	6	CSII/ s.c. injection	Ne
SE	M	29	13	5	s.c. injection	BR

Abbreviations: CSII: continuous subcutaneous insulin infusion; IVII: intravenous insulin infusion; PR: proliferative retinopathy; BR: background retinopathy; N: nephropathy; Ne: neuropathy; and M: macroangiopathy.

mine the content of nonenzymatically bound glucose of different serum protein fractions.

In a third group (group C) of 10 patients with IDDM an extensive longitudinal study was performed. These patients, all receiving a constant insulin infusion for a year or more, were selected because of proposed glycemic stability. Nine

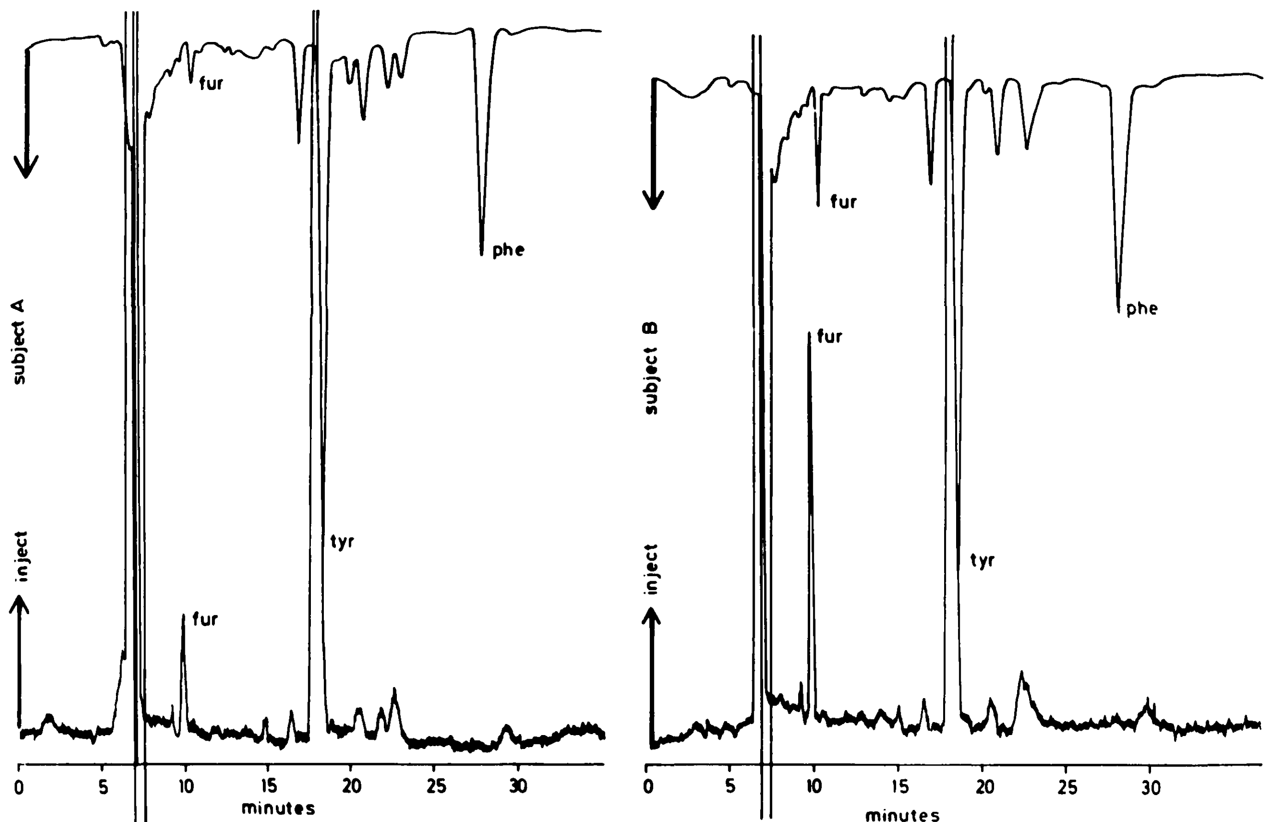

FIG. 1. HPLC-Chromatograms of whole serum hydrolysate from a normal (A) and a diabetic subject (B). The eluting compounds are detected by their UV absorbance at 254 and 280 nm (upper and lower trace, respectively). Furosine (fur), tyrosine (tyr), and phenylalanine (phe) are the major UV-absorbing substances.

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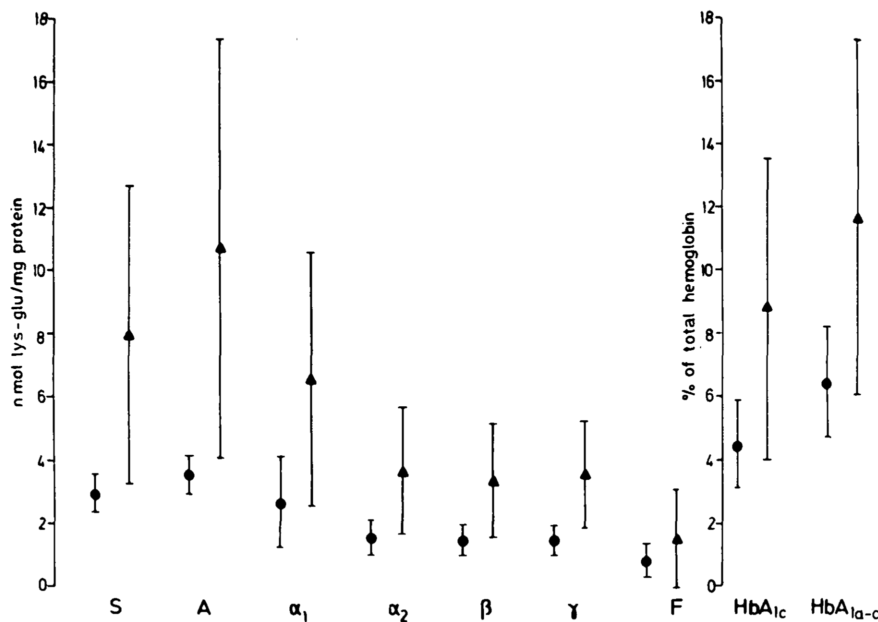

FIG. 2. *Nonenzymatic glycosylation of human serum proteins after electrophoretic separation of 20 normal individuals (●) and 20 diabetic patients (▲) (mean ± 2 SD). The proteins are whole serum (S), albumin (A), and the protein fractions α₁, α₂, β, γ as obtained by electrophoresis and fibrin peptides (F). Glycosylated hemoglobins are shown for comparison. For details see* METHODS.

of these patients were treated with an open-loop insulin delivery system (Cardiac Pacemakers Inc., Indianapolis, Indiana; Auto-Syringe pump, Auto-Syringe, Inc., Hooksett, New Hampshire; or Promedos, Siemens, Munich, FRG). Detailed clinical characteristics are described in Table 1. The patients were on a self-monitoring of blood glucose program and were urged to test blood glucose at least twice every day and 6–8 times on 1 day once a week with a reflectance meter (Glucose-meter, Wolff GmbH, Wuppertal, FRG) using glucose strips (Dextrostix, Ames GmbH, Frankfurt, FRG). Patients were seen at least every 4 wk and blood was taken for determination of lipids, blood glucose, free insulin, lys-GSP, and HbA_{1a-c}.

In a fourth group (group D) 60 subjects who were referred to the hospital as outpatients for a glucose tolerance test (OGTT), an oral glucose load of 75 g (N = 15, group D_1) or 100 g (N = 45, group D_2) was given after an overnight fast. Samples for lys-GSP were taken along with a sample for the glucose assay, all immediately before glucose administration.

Lys-GSP was also determined in two patients with insulinomas. The insulinomas were diagnosed by determination of blood glucose, C-peptide, and proinsulin during a prolonged fasting.

Determination of glycosylated serum proteins. Nonenzymatically (ε-N-lysine)-bound glucose yields furosine when hydrolyzed with 6 mol/L HCl.[15] This specific degradation product may be used for the determination of nonenzymatically glycosylated proteins. A detailed description applying this furosine method for the determination of glycosylated albumin has been published.[7] Briefly, serum proteins, either fractionated by preparative electrophoresis[14] or unfractionated serum (50 μl), were added to 1 ml of a solution of 1.6 g UO_2-acetate per liter isotonic saline. After centrifugation at 10,000 × g for 2 min the precipitated protein was dissolved in 0.5 ml 6 mol/L HCl and hydrolyzed for 18 h at 95°C. A synthetic fructose-lysine standard[7] equally treated was used for calibration. Then 30 μl of the diluted (1:10) hydrolysate was subjected to HPLC.

The chromatographic system consisted of a Solvent Delivery System, Model 6000A, an automated injector, Model WISP 710A, and a UV absorbance detector model 440 (all from Waters Assoc. Inc., Milford, Massachusetts). Two μ-Bondapak C_{18} columns (30 × 0.39 cm) were arranged in

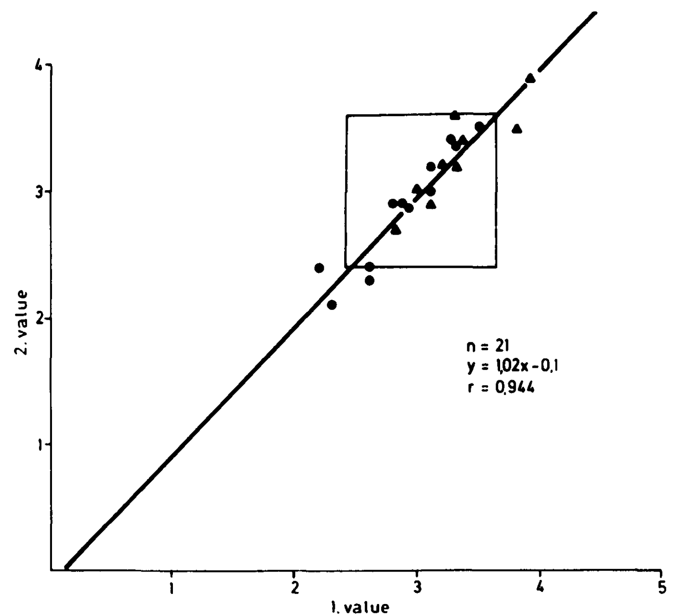

FIG. 3. *The intraindividual variance (2-wk interval) of lys-GSP is shown for 12 normal subjects (●) and 9 subjects with impaired glucose tolerance (▲). The framed area indicates the normal range of lys-GSP.*

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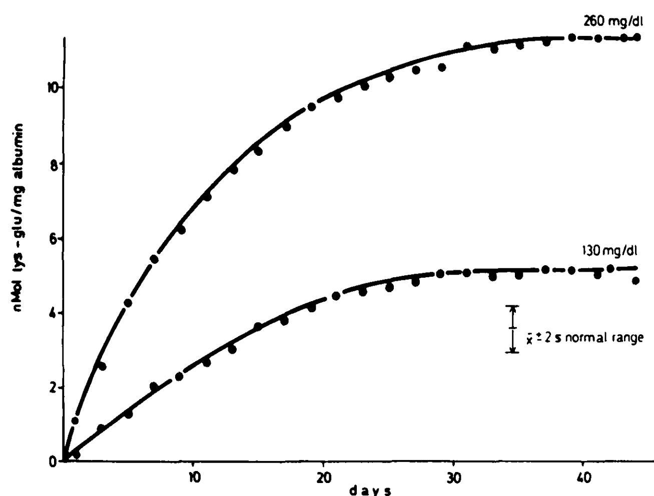

FIG. 4. In vitro glycosylation of human serum albumin under steady-state conditions. Experimental details are described in METHODS.

series; 0.63 g/L H_3PO_4 was used as the isocratic eluent. Absorption was recorded simultaneously at 254 and 280 nm and detector sensitivity was set at AUFS = 0.01. The amount of glycosylated serum proteins, calculated from the peak heights ratio of $furosine_{(sample)}$ versus $furosine_{(standard)}$, was expressed as nmol ε-aminolysine-bound glucose per mg protein (nmol lys-glu/mg protein), and related to the height of the phenylalanine peak of the sample, which served as an internal protein standard (Figure 1). When serum proteins were reduced by $NaBH_4$ before hydrolysis, the same elution pattern was obtained but lacked the furosine peak. Specificity of the determination was further assessed by incubating the serum with urea (20 g/L) or aspirin 1 mmol/L for 2 days at 37°C, but no change in furosine content was found. Free glucose up to 55 mmol/L added to serum just before hydrolysis did not interfere with the assay. The intra- and interassay precision of the method is given by coefficients of variation of 2.9% and 8.4%, respectively. Thus the method is rapid, specific, sensitive, and precise enough to meet laboratory standards for routine determinations.

To test the dependency of albumin glycosylation on glucose concentration with regard to the plasma half-life of albumin, the following in vitro experiment was performed: normal human serum albumin was incubated at 37°C in the presence of a "borderline" (130 mg/dl) and a "diabetic" (260 mg/dl) glucose concentration. To approach physiologic steady-state conditions the in vivo turnover of albumin (plasma half-life 10.5–20 days)[16] was simulated by daily replacement of 1/20 vol of the incubation mixture by a new mixture of the same composition, thus achieving a "half-life" of 13.8 days. Nonenzymatic glycosylation of albumin was determined as described before.

Determination of HbA_{Ia+b} and A_{Ic} was performed by the macrocolumn technique according to Trivelli et al.[11] HbA_{Ia-c} was measured by the thiobarbituric acid reaction as described by Gabbay et al.[12] and the results were correlated to the method cited above.

Blood glucose was determined by the glucose dehydrogenase assay.[17]

RESULTS

Evaluation of glycosylated serum proteins. HPLC chromatograms of serum hydrolysates of one healthy and one diabetic subject are demonstrated in Figure 1. While the phenylalanine peaks are comparable in height, reflecting the same protein content, the furosine peak is much higher in the chromatogram obtained from the diabetic subject. The lys-glu values of plasma protein fractions from 20 normal (from group A) and 20 diabetic subjects (from group B) are shown in Figure 2. Since the parameters are nearly normally dis-

TABLE 2

Mean HbA_{Ia-c}, lys-GSP, and averaged blood glucose levels from diabetic patients characterized in Table 1 (time course of the three parameters was followed up to 1 yr)

	HbA_{Ia-c} (%)			lys-GSP (nmol/mg)			Mean blood glucose (mg/dl) of the follow-up study		
Patient	N	x̄	SD	N	x̄	SD	N	x̄	SD
SR	15	7.3	1.2	14	5.3	1.0	47	132.0	40.1
NO	10	9.0	2.0	9	6.1	1.2	32	135.0	51.4
MH	12	8.9	1.2	12	6.7	1.2	48	153.0	57.3
SB	6	7.8	1.6	6	7.2	0.7	15	160.8	42.7
ZR	16	11.1	2.6	17	7.9	2.4	53	167.8	77.1
DR	10	7.5	1.4	10	5.8	0.8	18	174.4	46.5
FH	6	10.8	4.2	6	9.6	3.2	13	211.7	66.6
HP	5	8.0	2.9	7	5.4	2.2	14	129.8	44.4
SI	7	9.3	2.0	7	6.75	2.4	21	145.7	64.4
SE	3	9.3	2.8	5	7.0	0.35	10	184.1	47.3
Nondiabetic controls	52	6.4	0.9	52	3.0	0.3			

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tributed, the statistical evaluation (i.e., means ± 2 SD) is correct. Apart from the fact that the plasma proteins are glycosylated to a different extent (probably due to their different half-lives), it is clear from Figure 2 that in general normal and diabetic values of the glycosylated proteins show much less overlap than those of glycosylated hemoglobins. Figure 2 further indicates that albumin contains the highest amount of lysine-bound glucose and that it can discriminate well between nondiabetic and diabetic subjects. The comparison of lys-GSP and glycosylated albumin determined for 20 different sera from diabetic patients yielded a correlation coefficient of $r = 0.984$ and a slope of $y = 0.7x + 0.43$. Thus one can use the simpler determination of lys-GSP instead of albumin as a control parameter. To get some idea about the intraindividual variance, the determination of lys-GSP has been repeated in 21 subjects (from group D_1 and D_2) after a 2-wk interval. When first and second values are correlated, the regression line is $y = 1.02x + 0.1$, $r = 0.944$ (Figure 3).

Nonenzymatic glycosylation of albumin was studied in vitro at constant glucose concentrations but at "turnover" conditions for albumin with a half-life of 13.8 days. Details are described in METHODS. As illustrated in Figure 4 steady-state levels of 5.15 and 11.3 mmol lys-glu/mg albumin were established after about 40 days at 130 and 260 mg glucose/dl, respectively. The average value of lys-glu is 3.7 nmol/mg albumin in normal subjects (Figure 2). On extrapolation from Figure 4 this would correspond to an average blood sugar concentration of about 90 mg/dl, consistent with the average blood sugar level of nondiabetic subjects.[18]

Follow-up of lys-GSP, HbA_{1a-c}, and glucose levels in diabetic patients. In 10 diabetic patients (group C) characterized in Table 1, lys-GSP, HbA_{1a-c}, and averaged blood glucose levels were followed from several months up to 1½ yr. The means of the whole group are summarized in Table 2. For better illustration of the individual time course three examples are shown in Figure 5 (A–C), where blood glucose values are given as the means of 6–8 determinations performed on the respective days. The blood sugar values in Table 2 represent the means calculated from the whole follow-up study. In no case was complete normalization of the glucose balance achieved. This is also clearly reflected by the lys-GSP levels, which remained elevated in each patient. HbA_{1a-c}, however, appeared to be normalized in 4 of 10 patients (Table 2). The correlation of lys-GSP and HbA_{1a-c} is shown in Figure 6. The correlation of HbA_{1a-c} with blood glucose was $r = 0.615$, $y = 0.026 + 4.04$, while that of lys-GSP with blood glucose was significantly ($P < 0.05$) better, with an r-value of 0.878, $y = 0.04x + 0.25$. As shown in Figure 7 the regression line fitted quite well the theoretical curve passing the zero point and the mean of the normal range. The mean blood glucose values shown in Figure 7 correspond to the average level of glycemia calculated from periods (4 wk for lys-GSP; 3 mo for HbA_{1a-c}) with rather constant blood glucose values.

Two patients with fasting hypoglycemia due to insulinomas displayed lys-GSP levels below the normal range, i.e., 2.4 and 2.0 nmol lys-glu/mg protein. After surgical removal of the insulinoma, lys-GSP of the latter patient had risen after

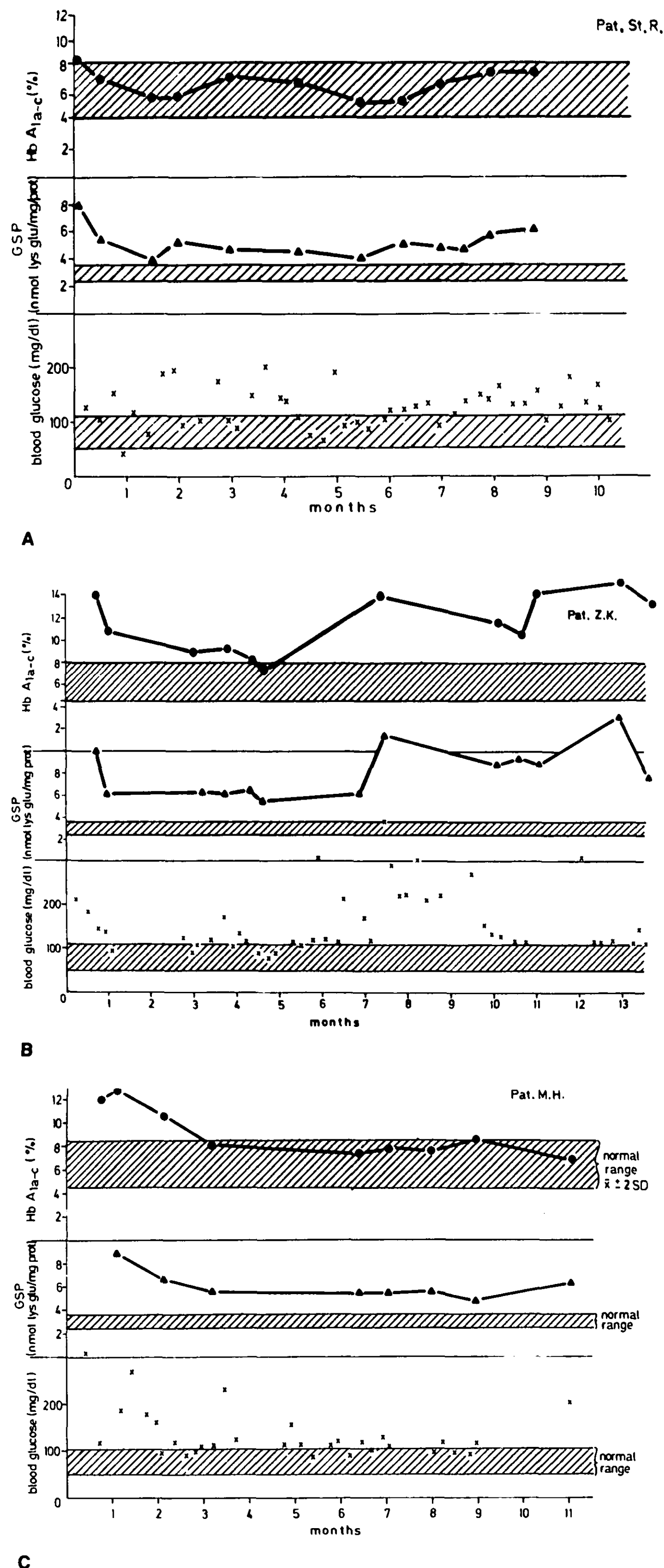

FIG. 5A–C. Lys-GSP (▲), HbA_{1a-c} (●), and mean blood glucose (x) values in a longitudinal study. The time course is shown for three distinct diabetic patients (St. R., Z. K., M. H.). For further details see text.

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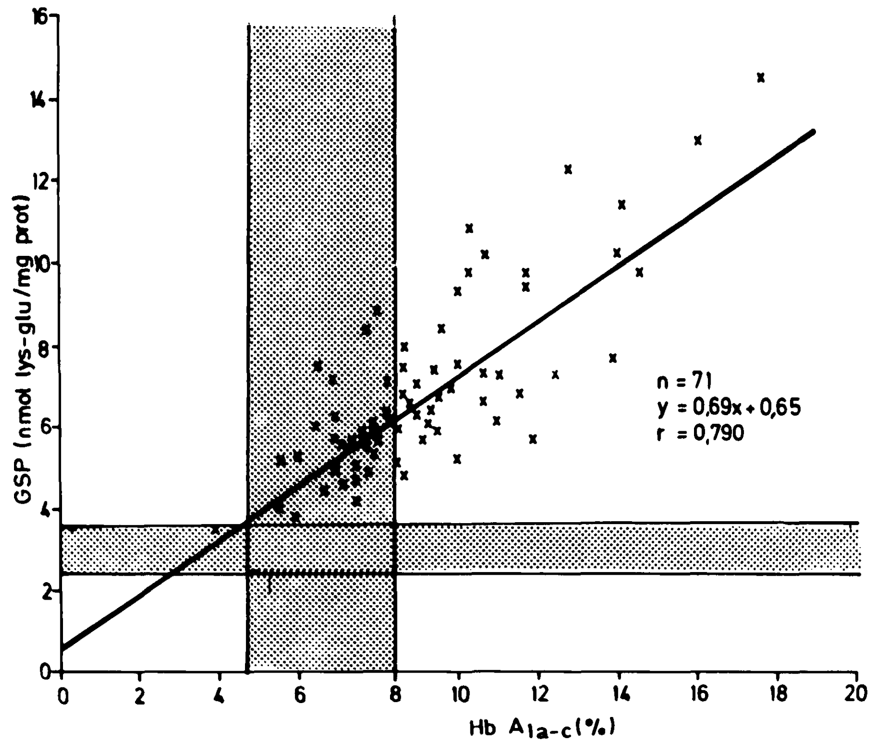

FIG. 6. The relationship of lys-GSP to HbA_{1a-c} is shown for 71 diabetic subjects (group B). Shaded areas represent normal ranges (mean ± 2 SD).

4 wk to 3.4 nmol/mg protein, well within the normal range. We had no opportunity to measure lys-GSP after surgery of the first patient.

Lys-GSP in subjects with oral glucose tolerance tests (OGTT). To test the discriminative power of lys-GSP in subjects with different glucose tolerances, 60 OGTT were

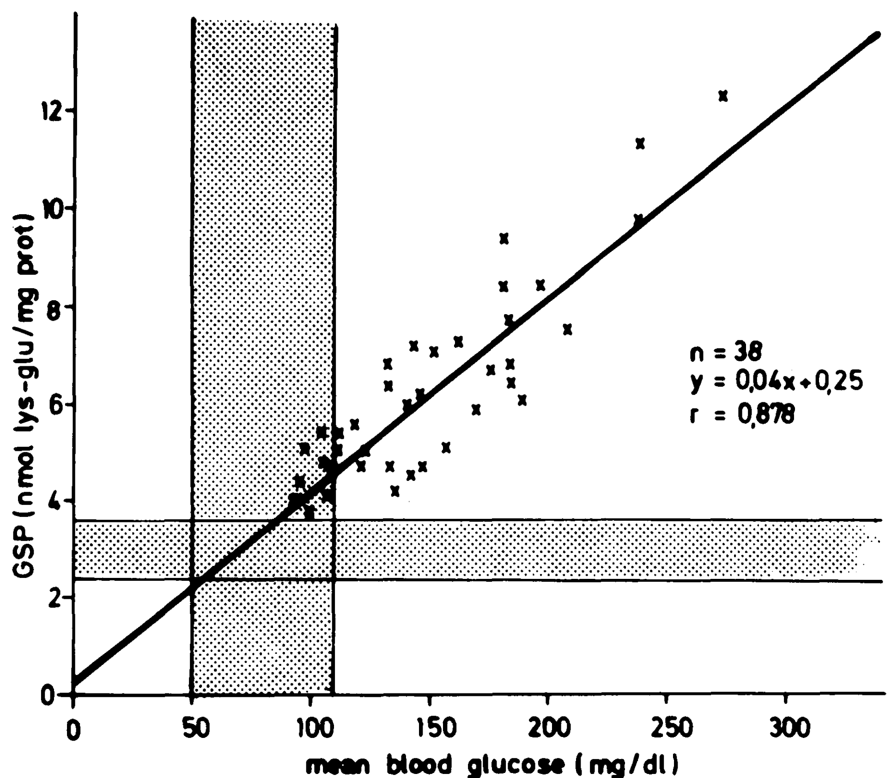

FIG. 7. The relationship of lys-GSP to mean blood glucose is shown for diabetic patients (group C). Mean blood glucose values were obtained by averaging the daily mean glucose levels (N = 6–8) determined once a week for 4 wk. Shaded areas represent normal ranges (mean ± 2 SD).

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performed (see Figure 8). Results were assessed according to the criteria of the National Diabetes Data Group (NDDG).[13] It should be noted that the criteria of the NDDG, although designed for a 75-g glucose load, were also applied to the group who received 100 g glucose (group D_2). Of group D_1 8/15 and of group D_2 27/45 were classified as normal or nondiagnostic.[13] All had normal lys-GSP levels. From the 4 subjects of group D_1 with impaired glucose tolerance 3 had normal and 1 had elevated lys-GSP values, while in group D_2 8/10 with impaired glucose tolerance had normal and 2/10 had elevated lys-GSP. All three patients of group D_1 classified as having diabetes had increased lys-GSP. In group D_2, however, only 3 of 8 patients classified as having diabetes had elevated lys-GSP, whereas 5/8 had normal lys-GSP. During further clinical observations for 3 wk these 5 "diabetic" patients exhibited no urinary glucose excretion, fasting blood sugar levels below 120 mg/dl, and postprandial glucose concentrations that were normal or close to normal. These 5 patients were therefore classified as having impaired glucose tolerance. The diagnosis of diabetes in the remaining 3 patients of group D_2 was confirmed by further clinical observations. This is also true for the 3 diabetic patients of group D_1.

DISCUSSION

The results presented here extend our former studies on nonenzymatic glycosylation of proteins in vivo and in vitro.[2,7] Our studies on the various serum protein fractions separated on electrophoresis revealed that all are more or less susceptible to nonenzymatic glycosylation and present an increase in lysine-bound glucose in diabetic patients. For long-term assessment of the average blood glucose level, glycosylated albumin or, owing to the high albumin content of serum, lys-GSP appears most suitable. As compared with HbA_{1a-c}, lys-GSP offers the following advantages: (1) there is very little overlap of the lys-GSP values of nondiabetic and diabetic subjects (Figure 2); (2) the intraindividual variation of lys-GSP in normal subjects is very small (less than 5%, Figure 3); (3) there is better correlation of lys-GSP ($r = 0.878$) than of HbA_{1a-c} ($r = 0.615$) with mean blood glucose in hyperglycemic patients (Figure 7); (4) a normal HbA_{1a-c} value does not necessarily reflect normoglycemia (Table 2 and Figure 5); and (5) due to the shorter half-life of albumin, lys-GSP follows changes of glycemia faster than HbA_{1a-c} (Table 2 and Figure 5).

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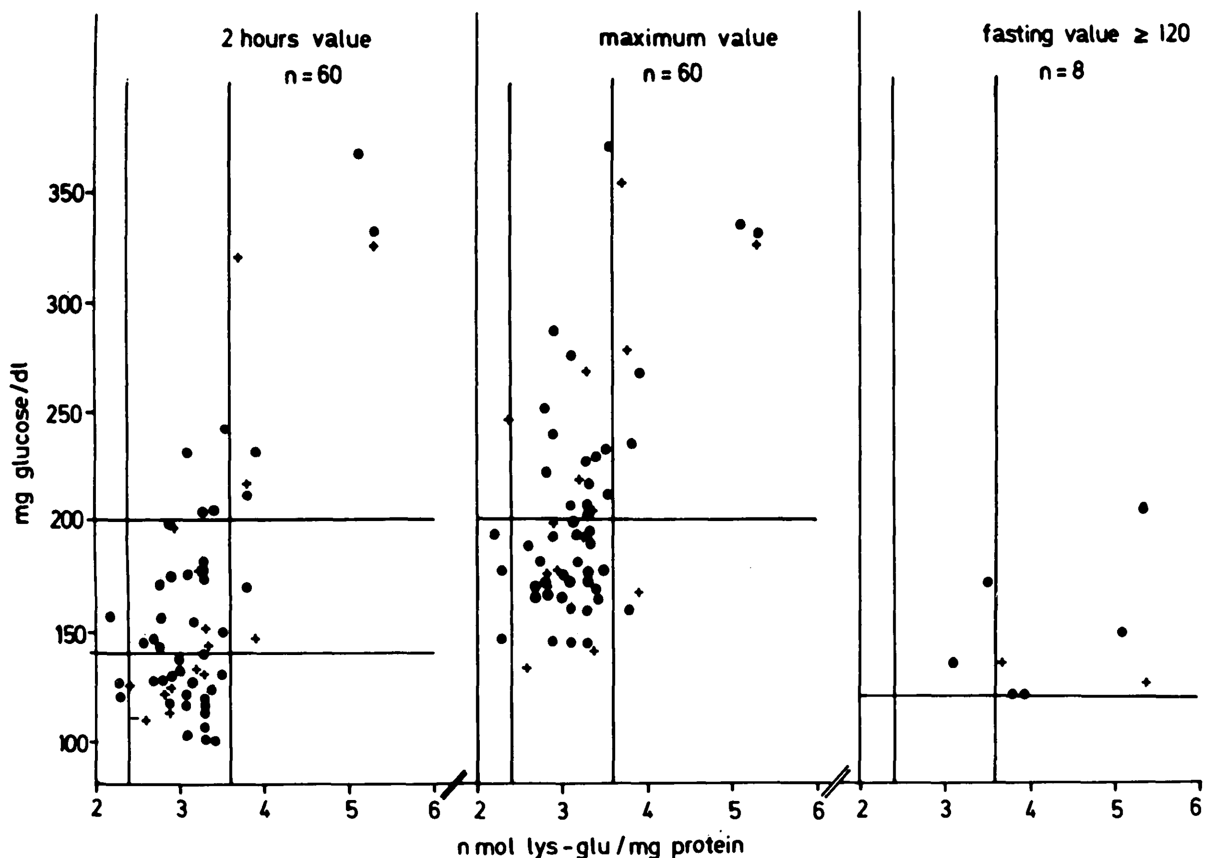

FIG. 8. The distribution of lys-GSP values relative to the glucose levels of 60 OGTT is shown (group D_1 + D_2). Criteria of the National Diabetes Data Group[13] are used to assess the results of the OGTT. A 75-g (+) or 100-g (●) glucose load was given.

Recently, Mehl et al.[19] compared the diagnostic value of glycosylated serum proteins (GSP) and glycosylated hemoglobins (HbA_{1a-c}) in patients with IDDM, both parameters measured as hydroxymethylfurfural (HMF). Similar to our results, mean blood glucose values were well correlated to GSP as well as to HbA_{1a-c}. However, 25% of their diabetic patients (IDDM) had normal GSP values but elevated HbA_{1a-c}, at variance with our findings.

Other investigators[5,20] using the HMF method found that in their diabetic patients the relative increase was greater for GSP than for HbA_1, suggesting that glycosylated serum proteins may be a more sensitive indicator of integrated blood glucose. Determination of GSP clearly separated a group of control subjects from a group of diabetic patients. These findings are consistent with our results. As pointed out by Guthrow et al.,[1] GSP may have advantages over HbA_1 measurements in some clinical situations such as presence of HbF, pregnancy, or hemolytic diseases because of altered erythrocyte lifetime.

A single determination of lys-GSP clearly discriminates the normal and diabetic glucose tolerance. About 20% of the patients classified as having impaired glucose tolerance showed slightly elevated lys-GSP. Since 6/9 of the patients with increased lys-GSP values had diabetes, the predictive value of the lys-GSP determination for diabetes is near 70%. On the other hand, an elevated lys-GSP value clearly shows that these subjects have either impaired glucose tolerance or diabetes. Most of the patients classified from the OGTT to have impaired glucose tolerance showed normal lys-GSP, suggesting that they were normoglycemic under usual life conditions, but had elevated blood sugar during a provocative test such as OGTT. Accordingly, lys-GSP, like glycosylated hemoglobin (HbA_1) cannot be used to detect impaired glucose tolerance.[21–28]

Not only blood proteins but also other proteins of insulin-insensitive tissue are glycosylated higher in diabetes compared with normal subjects (for review see ref. 29). Recently, it was shown that an index of late complications correlates fairly well with nonenzymatic glycosylation (NEG) of aortic tissue,[30] suggesting that the extent of NEG of tissue proteins may be related to the extent and severity of the late complications. Since mean blood glucose correlates with NEG of aortic tissues[30] and also with lys-GSP (this study), the determination of the latter allows the possibility to assess indirectly the degree of NEG of proteins of insulin-insensitive tissues. A study is under way in this laboratory to compare metabolic control as judged by blood glucose, HbA_1, and lys-GSP with the development of late diabetic complications.

ACKNOWLEDGMENTS: This work contains part of the doctoral thesis of E.R.

We thank E. Wagner for excellent technical assistance and D. Lehmeier for typing the manuscript.

This work was supported by the Bundesministerium für Forschung und Technologie and by the Deutsche Forschungsgemeinschaft, Bad Godesberg, FRG.

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REFERENCES

[1] Guthrow, C. E., Morris, M. A., Day, J. F., Thorpe, S. R., and Baynes, J. W.: Enhanced nonenzymatic glycosylation of human serum albumin in diabetes mellitus. Proc. Natl. Acad. Sci. USA 1979; 76:4258–68.

[2] Dolhofer, R., and Wieland, O. H.: Increased glycosylation of serum albumin in diabetes mellitus. Diabetes 1980; 29:417–22.

[3] Ney, K. A., Colley, K. J., and Pizzo, S. V.: The standardization of the thiobarbituric assay for non-enzymatic glucosylation of human serum albumin. Anal. Biochem. 1981; 118:294–300.

[4] Yue, D. K., Morris, K., McLennan, S., and Turtle, J. R.: Glycosylation of plasma protein and its relation to glycosylated hemoglobin in diabetes. Diabetes 1980; 29:296–300.

[5] McFarland, K. F., Catalano, E. W., Day, J. F., Thorpe, S. F., and Baynes, J. W.: Nonenzymatic glucosylation of serum proteins in diabetes mellitus. Diabetes 1979; 28:1011–14.

[6] Kennedy, L., Mehl, T. D., and Merimee, T. J.: Nonenzymatically glycosylated serum protein in diabetes mellitus, an index of short-term glycemia. Diabetologia 1981; 21:94–98.

[7] Schleicher, E., and Wieland, O. H.: Specific quantification by HPLC of protein (lysine) bound glucose in human serum albumin and other glycosylated proteins. J. Clin. Chem. Clin. Biochem. 1981; 19:81–87.

[8] Dolhofer, R., and Wieland, O. H.: Glycosylation of serum albumin: elevated glycosyl-albumin in diabetic patients. FEBS Lett. 1979; 103:282–86.

[9] Mayer, T. K., and Freedman, Z. R.: Protein glycosylation in diabetes mellitus: a review of laboratory measurements and of their clinical utility. Clin. Chim. Acta 1983; 127:147–84.

[10] Schneck, A. G., and Schroeder, W. A.: The relation between minor components of whole normal human adult hemoglobins as isolated by chromatography and starch block electrophoresis. J. Am. Chem. Soc. 1961; 83:1472–78.

[11] Trivelli, L. A., Ramsey, H. M., and Lai, H. T.: Hemoglobin components in patients with diabetes mellitus. N. Engl. J. Med. 1971; 284:353–57.

[12] Gabbay, K. H., Sosenko, J. M., Banudi, G. A., Mininsohn, M. J., and Flückiger, R.: Glycosylated hemoglobins: increased glycosylation of hemoglobin A in diabetic patients. Diabetes 1979; 28:337–40.

[13] National Diabetes Data Group: Classification and diagnosis of diabetes mellitus and other categories of glucose intolerance. Diabetes 1979; 28:1039–57.

[14] Wieland, O. H., Dolhofer, R., and Schleicher, E.: Glucosylation of hemoglobin and of serum albumin in diabetes. Diabetes 1979. Internat. Congr. Series 500. Waldhausl, W. K., Ed. Elsevier, North Holland, 1979:721–27.

[15] Finot, P. A.: *In* Protein in Human Nutrition. Porter, J. W. G., and Rolls, B. A., Eds. New York, Academic Press, 1973:501.

[16] Schultze, H. E., and Heremans, J. F.: Molecular Biology of Human Proteins, Vol. 1. New York, Elsevier, 1966:212.

[17] Dolhöfer, R., Weiss, L., and Wieland, O. H.: Experience with the glucose-dehydrogenase-UV-method for the determination of blood glucose. J. Clin. Chem. Clin. Biochem. 1976; 14:415–17.

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[18] Malherbe, C., DeGasparo, M., DeHertogh, R., and Hoet, J. J.: Circadian variations of blood sugar and plasma insulin levels in man. Diabetes 1979; 28:1039–57.

[19] Mehl, T. D., Wenzel, S. E., Russell, B., Gardner, D., and Merimee, T. J.: Comparison of two indices of glycemic control in diabetic subjects: glycosylated serum protein and hemoglobin. Diabetes Care 1983; 6:34–39.

[20] Gragnoli, G.: Non-enzymatic glycosylation of serum proteins as an indicator of diabetic control. Diabetologia 1982; 22:222–23.

[21] Dods, R. F., and Bolmey, C.: Glycosylated hemoglobin assay and oral glucose tolerance test compared for detection of diabetes mellitus. Clin. Chem. 1979; 25:764–68.

[22] Dix, D., Cohen, P., Kinsley, S., Senkbeil, J., and Sexton, K.: Glycohemoglobin and glucose tolerance tests compared as indicators of borderline diabetes. Clin. Chem. 1979; 25:877–79.

[23] Dunn, P. J., Cole, R. A., Soeldner, J. S., and Gleason, R. E.: Reproducibility of hemoglobin A_{1c} and sensitivity to various degree of glucose intolerance. Ann. Intern. Med. 1979; 91:390–96.

[24] Bolli, G., Compagnucci, P., Cartechini, M. G., et al.: Hemoglobin A_1 in subjects with abnormal glucose tolerance but normal fasting plasma glucose. Diabetes 1980; 29:272–77.

[25] Lev-Ran, A., and Vanderlaan, W. P.: Glycohemoglobins and glucose tolerance. JAMA 1979; 241:912–14.

[26] Lev-Ran, A.: Glycohemoglobin: its use in the follow-up of diabetes and diagnosis of glucose intolerance. Arch. Intern. Med. 1981; 141:747–49.

[27] Pettitt, D. J., Knowler, W. C., Lisse, J. R., and Bennett, P. H.: Development of retinopathy and proteinuria in relation to plasma-glucose concentrations in Pima Indians. Lancet 1980; 2:1050–52.

[28] Verillo, A., DeTeresa, A., and Nunziata, V.: The relationship between glycosylated haemoglobin levels and various degree of glucose intolerance. Diabetologia 1983; 24:391–93.

[29] Wieland, O. H.: Protein modification by non-enzymatic glucosylation: possible role in the development of diabetic complications. Mol. Cell. Endocrinol. 1983; 29:125–31.

[30] Vogt, B., Schleicher, E., and Wieland, O. H.: ε-Amino-lysine-bound glucose in human tissues obtained at autopsy: increase in diabetes mellitus. Diabetes 1982; 31:1123–27.

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