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Different Behaviour of Haemoglobin A_{1a-c} and Glycosyl-Albumin Levels During Recovery from Diabetic Ketoacidosis and Non-acidotic Coma

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Summary. Glycosylated haemoglobin (HbA_{1a-c}) and serum albumin (glycosyl-albumin) have been determined in patients with severe diabetic ketoacidosis and non-acidotic coma. Within one week of therapy the level of glycosyl-albumin decreased from 184 mmol 5-hydroxymethylfurfural (HMF)/mol albumin to 152 mmol HMF/mol albumin ($p < 0.01$) and was gradually lowered by some 40% during a period of 17 days. In contrast, the level of HbA_{1a-c} remained unchanged. From these observations and findings in a patient with insulinoma, it appears that glycosyl-albumin provides a more acute measure of variation in relative glycaemia than HbA_{1a-c}, and may prove useful as a measure of medium-term diabetes control.

Key words: Diabetes control, glycosyl-albumin, haemoglobin A_{1a-c}, non-enzymatic glycosylation, diabetic ketoacidosis, hyperglycaemia.

The glycosylated forms of haemoglobin, designated haemoglobins A_{1a-c} (HbA_{1a-c}), are increased in patients with imperfectly controlled diabetes mellitus [1]. Recently, serum albumin has been shown to be subject to non-enzymatic glycosylation [2–6] and to be elevated in the serum of diabetic patients [2–4, 6]. Little is known of the temporal relationship between the changes in the levels of blood glucose and glycosylated albumin. Patients suffering from diabetic coma offer the possibility of investigating the interdependence of fluctuations in the blood glucose and glycosyl-protein levels. The present study was designed to examine these interrelations in such patients over the course of more than 2 weeks.

Patients

Studies were performed on 12 diabetic patients suffering from ketoacidosis or non-acidotic coma. Clinical data are shown in Table 1. The patients were treated with low dose intramuscular or continuous IV infusion of insulin [7, 8]. In cases of severe ketoacidosis the amount of IV infused insulin was adapted to the changing states of insulin resistance by reducing infusion rates step by step with falling blood glucose levels as described by Renner et al. [9]. Patients with milder ketoacidosis (pH > 7.1) or non-acidotic coma were treated with low dose insulin administration from the very beginning. Some of the patients received in addition antibiotics, digoxin, diuretics, vitamins or antihypertensive drugs. None of the patients received blood transfusions at any time. We also had the opportunity to investigate a woman suffering from insulinoma before and after surgery.

Materials

Bio-Rex 70 and Affi-Gel Blue were from Bio-Rad, Munich, FRG. Whatman DE 52 Cellulose was obtained from Hormuth & Vetter, Wiesloch, FRG. 2-Thiobarbituric acid, test-kit "System Gluc-DH UV-Test" and all other chemicals were from Merck, Darmstadt, FRG.

Methods

Blood glucose was measured by the glucose-dehydrogenase ultraviolet method [10]. Protein was determined by the biuret method [11]. Haemoglobin was assayed by the method of van Kampen & Zijlstra [12]. For HbA_{1a-c} and glycosyl-albumin analyses heparinized blood was centrifuged at 3000×g for 10 min immediately after withdrawal. The plasma was carefully removed and stored at −25 °C for up to 4 weeks. The erythrocytes were washed three times with 0.9% NaCl, before haemolysis by mixing one volume of packed cells with two volumes distilled water. The haemolysate was dialyzed against developer No. 6 [13] for 18 h at room temperature and stored at −25 °C for up to 4 weeks.

HbA_{1a-c} were determined by the macrocolumn method (column size 1 × 30 cm) by the method of Schnek and Schroeder [14] in the modification of Trivelli et al. [13] and expressed as percentage of total haemoglobin. Albumin from human plasma was purified (> 99%) by chromatography on DEAE-cellulose and

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Table 1. Admission details of the diabetic coma patients

Patients	Age (years)	Sex	Duration of diabetes (years)	Previous treatment	Conscious state	Venous pH	Blood glucose (mmol/l)	HbA_{1a-c} (%)	Glycosyl-albumin $\left(\frac{\text{mmol HMF}^b}{\text{mol albumin}}\right)$
Ketoacidotic									
1	16	M	ND[a]	None	Unconscious	7.00	66.9	15.3	
2	21	M	12	Insulin	Unconscious	6.74	62.4	15.1	171
3	74	M	12	Insulin	Unconscious	6.84	53.4	13.8	
4	64	F	16	Insulin	Normal	7.15	51.8	12.9	
5	71	F	3	Oral antidiabetics	Unconscious	7.07	49.2	20.6	
6	32	F	15	Insulin	Drowsy	6.84	46.1	17.6	151
7	61	F	1	Insulin	Normal	7.19	38.0	20.7	208
8	15	M	2	Insulin	Drowsy	7.01	37.7	19.8	208
9	15	M	ND	None	Normal	7.32	30.1	15.3	271
10	73	F	8	Insulin	Drowsy	7.05	28.3	14.9	
Hyperglycaemic non-ketotic									
11	45	M	ND	None	Unconscious	7.4	49.4	17.2	150
12	83	M	ND	None	Drowsy	7.38	44.1	18.4	132
Mean							46.5	16.8	184
SEM							3.4	0.8	18

[a] newly diagnosed
[b] 5-hydroxymethylfurfural

Table 2. Behaviour of the levels of haemoglobins A_{1a-c} (A) and glycosyl-albumin (B) during recovery from diabetic ketoacidosis and non-acidotic hyperglycaemia. Results in A from patients 1–12 (Table 1), results in B from patients 2, 6, 7, 8, 9, 11, 12 (Table 1). The decrease in HbA_{1a-c} in the group identical to that studied in B was from 17.7 ± 0.8 to 16.8 ± 0.7%

	A (n = 12)		B (n = 7)	
Days of therapy	Haemoglobins A_{1a-c} (%)	Glucose (mmol/l)	Glycosyl-albumin $\left(\frac{\text{mmol/HMF}^a}{\text{mol albumin}}\right)$	Glucose (mmol/l)
1	16.8 ± 0.8	33.1 ± 3.4	184 ± 18	29.7 ± 3.8
2		14.8 ± 1.3	184 ± 23	13.1 ± 1.2
3	16.5 ± 0.7	13.0 ± 1.2	180 ± 20	11.7 ± 0.9
4		11.8 ± 1.0		10.3 ± 1.1
5	15.8 ± 0.8	8.6 ± 0.6		9.0 ± 0.6
6		11.6 ± 1.1		10.5 ± 1.4
7	15.9 ± 0.7	9.7 ± 1.3	152 ± 13[b]	10.9 ± 1.5

Results are given as mean ± SEM
[a] 5-hydroxymethylfurfural
[b] $p < 0.01$

Affi-gel blue as described previously [2, 3]. The amount of glycosyl-albumin was determined by the thiobarbituric acid assay for the 5-hydroxymethylfurfural (HMF) [15] released after 24 h hydrolysis in acetic acid 2 mol/l [16] at 92 °C as described previously [3].

The interassay coefficients of variation were 6.3% (n = 33) in the HbA_{1a-c} and 5.3% (n = 6) in the glycosyl-albumin determinations. Results are given as mean ± SEM. Statistical significance in Table 2 and Figure 1 was calculated using Student's t-test for paired data.

Results

As indicated in Table 1 the average initial levels of HbA_{1a-c} and of glycosyl-albumin in the patients under study amounted to 16.8% and 184 mmol HMF/mol albumin, respectively, i.e. an almost threefold increase compared with our normal values of 6.5% HbA_{1a-c} [17] and 64 mmol HMF/mol albumin [3].

Table 2 shows the changes in HbA_{1a-c}, glycosyl-albumin and blood glucose during the first week of therapy. As can be seen, there was a non-significant reduction of HbA_{1a-c} by 5%, whereas the level of glycosyl-albumin was significantly lowered by 17% within the same period. It appears therefore that the two glycosylated proteins respond at different rates to a lowering of the blood glucose level. This came out more clearly when the behaviour of the two glycosyl-proteins was followed for a longer period. Thus, as illustrated in Figure 1B, the level of glycosyl-albumin continued to decline markedly up to day 17 while only a small change of HbA_{1a-c} was observed over the same interval (Fig. 1A). It should be noted that the control of glycaemia was comparable in both groups.

The results obtained from a diabetic patient with highly unstable blood glucose levels are shown in Fig. 2. No significant change in glycosyl-albumin (nor in HbA_{1a-c}) occurred in this patient, whose blood glucose level showed marked fluctuations during the 19 days of observation.

Regarding the temporal relationship between the onset of hyperglycaemia and the augmentation of HbA_{1a-c} and glycosyl-albumin, we had an opportunity to study a patient suffering from an insulinoma. As illustrated in Fig. 3 a biphasic increase in blood glucose concentration occurred in this patient during and after the removal of the tumour. The first transient elevation of blood glucose (peak value = 9.3 mmol/l) lasting for about 6 h was not associated with a detectable change in the amount of glycosyl-albumin. Two days later, however, after a longer phase (17 h) of hyperglycaemia, during which a maximum of 15.0 mmol/l was reached, glycosyl-albumin was markedly elevated. In contrast, the amount of HbA_{1a-c} remained essentially constant.

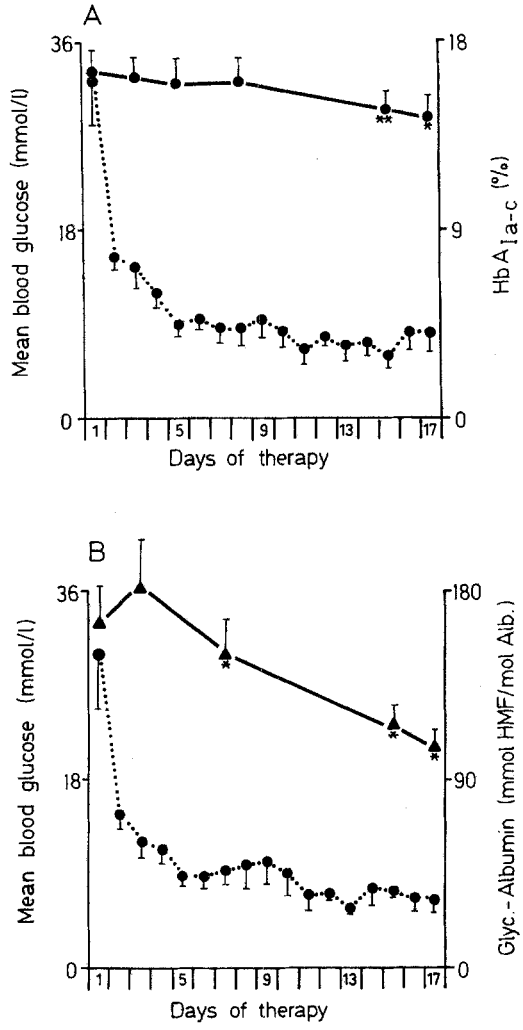

Fig. 1 A and B. Behaviour of the levels of **A** haemoglobins A_{1a-c} and **B** glycosyl-albumin during recovery from diabetic ketoacidosis and non-acidotic hyperglycaemia. Patients included in **A**: 1, 2, 4, 7, 11, 12; in **B**: 2, 7, 11, 12 (Table 1). In those subjects in **A** also studied in **B**, the decrease in HbA_{1a-c} was from 17.9 ± 1.2 to 14.8 ± 1.1%. Results are given as mean ± SEM. $*p < 0.01$; $**p < 0.025$. ●-----● Mean blood glucose; ●——● Haemoglobins A_{1a-c} (HbA_{1a-c}); ▲——▲ Glycosyl-albumin (Glyc.-Albumin). HMF = 5-hydroxymethylfurfural

Discussion

Compared with the values in our previous group of diabetics [3, 17], uncontrolled diabetic ketoacidosis and non-acidotic hyperglycaemia were accompanied by much higher levels of glycosyl-proteins. This suggested a way of investigating whether glycosyl-albumin might be a more flexible integrative indicator of diabetic control than HbA_{1a-c}. We therefore compared the effect of coma therapy on the levels of both glycosyl-proteins. Our results clearly indicate that glycosyl-albumin measurements employing highly purified albumin preparations [3] reflect the amelioration of the diabetic control more rapidly than does HbA_{1a-c}. Moreover they suggest that normalization of blood glucose for only short periods of time (i. e. 2 or 3 days) is not sufficient to decrease glycosyl-albumin (Fig. 2). The usefulness of HbA_{1a-c} in monitoring long-term control of glycaemia has recently been disputed in view of several reports indicating that glycohaemoglobin levels may fall after correction of hyperglycaemia much more rapidly than expected [18–26]. No such rapid changes of HbA_{1a-c} could be observed in the present study. The finding of rapid changes depends on the exact technique of HbA_{1a-c} determination by column chromatography (unpublished observations). Under the conditions used in the present study, the stable rather than the labile form of glycosylated haemoglobin is assayed. Concerning the measurement of glycosylated albumin it is noteworthy that the thiobarbituric acid assay reacts only with the stable ketoamine form of glycosyl-proteins, and not with the Schiff base [16].

The observations on the patient with an insulinoma suggest that glycosyl-albumin is formed more rapidly than it is eliminated from the blood stream. Furthermore, they are consistent with our view that glycosyl-albumin may represent a more appropriate indicator of diabetic control than HbA_{1a-c} since it appears to be more acutely related to blood glucose levels. Day et al. [27] recently reported that alloxan-diabetic rats show an increase in gly-

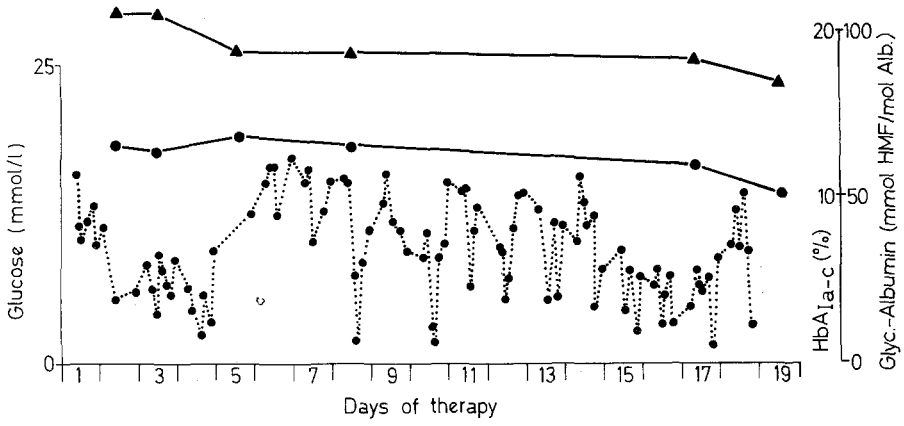

Fig. 2. Haemoglobins A_{1a-c} and glycosyl-albumin in a patient displaying highly unstable blood glucose levels. ●-----● Blood glucose; ●—● Haemoglobins A_{1a-c} (HbA_{1a-c}); ▲—▲ Glycosyl-albumin (Glyc.-Albumin). HMF = 5-hydroxymethylfurfural

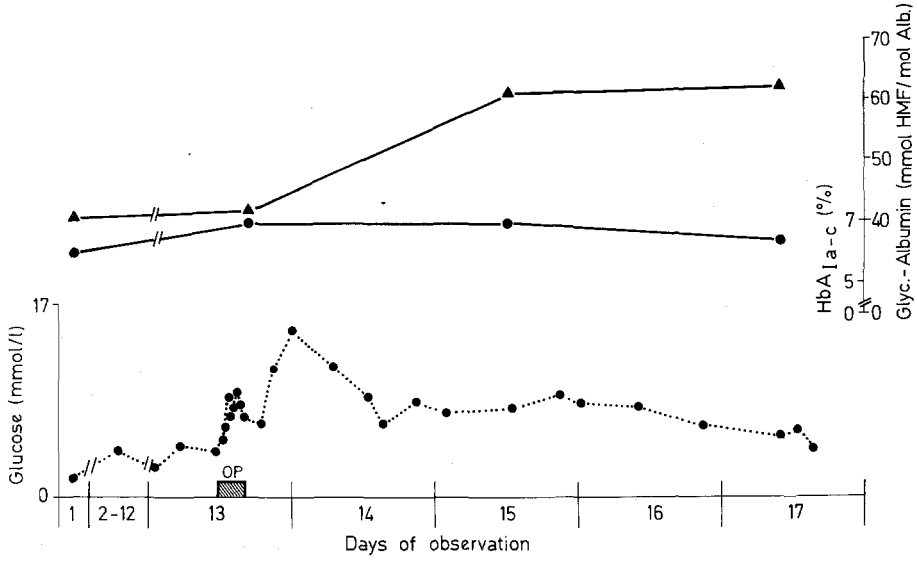

Fig. 3. Levels of blood glucose, haemoglobins A_{1a-c} and glycosyl-albumin in a patient with insulinoma. ●-----● Blood glucose; ●—● Haemoglobins A_{1a-c} (HbA_{1a-c}); ▲—▲ Glycosyl-Albumin (Glyc.-Albumin). OP indicates the time of the removal of the tumour. Ten determinations of HbA_{1a-c} and glycosyl-albumin between days 17 and 49 showed essentially no changes; blood glucose was also normal in this period

cosylated serum-proteins which is normalized within 2–3 days by insulin therapy. This finding, however, is not in conflict with our results, since the half-life time of rat albumin is shorter than that of human serum albumin by a factor of ten [28].

A possible objection to the use of glycosyl-albumin as a monitoring parameter of diabetes control is that a number of substances known to be bound by albumin might influence glycosylation of albumin. In order to get some information on this point we have studied a number of the drugs *in vitro* taken by our diabetic patients. When tested in therapeutic concentrations, essentially no influence was exerted by insulin, digoxin, β-methyl-digoxin, isosorbide dinitrate, frusemide, diazepam, trimethoprim, sulfamethoxazole, chloramphenicol, azlocillin, doxycyclin, or vitamins. Other sugars like mannose, galactose, fructose, and ribose were able to form glycosyl-albumin *in vitro* when studied over a concentration range of 6.9 to 55.5 mmol/l (data not shown).

Taken together, our results indicate that measurement of glycosyl-albumin might prove valuable in investigating the relationship between the development of late diabetic complications and diabetic control. So far, widespread application has been hampered by the laborious technique involved, but we have now developed a simple and rapid method which should facilitate further studies [29].

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