I use the Delta pump [3], an open loop insulin delivery system which utilizes U-100 regular insulin contained in a reservoir and propelled forward through the tubing by the repetitive action of a solenoid plunger pinching the area of tubing located between two check valves. The reservoir is usually refilled every 3–7 days depending on the patient's daily insulin requirement. Since the aggregation of insulin solutions had been described in implanted devices maintained at body temperature, I was surprised to meet this problem in the Delta open-loop pump. The precipitation seemed to start in either the tubing near the needle and/or in the tubing between the check valves. Since the tubing adjacent to the subcutaneously placed needle is pressed against the skin, aggregation here is consistent with the association of this problem with higher temperatures [4]. The insulin contained in the area between the valves is subjected to the repetitive action of the solenoid plunger. Aggregation here is consistent with the association of this problem with agitation of the insulin solution [4].

To circumvent this problem, I initially dissolved glutamic acid (50 mg/10 ml vial) into the insulin under sterile conditions [5]. Although this prevented the aggregation, dissolving the dicarboxylic amino acid was difficult and some patients complained of burning at the needle site, especially during the administration of the preprandial bolus. After publication of reference 2, I used a physiological concentration of NaHCO_3 (26 mmol/l) to achieve the same end. To obtain this concentration, which is equivalent to 0.26 mmol/10 ml vial, 0.3 ml of the commercially available (at least in the USA) bicarbonate solution (44.5 mmol/50 ml) is added under sterile conditions to a 10 ml vial of U-100 regular insulin. The addition of bicarbonate has been successful in preventing aggregation of the insulin solutions in the eight patients in whom this method has been used. The first patient was started nearly one year ago. Since aggregation seems to occur more readily when flow rates are low, it is particularly gratifying that this approach has been successful in a patient receiving 0.003 ml/h (0.3 U/h).

Insulin aggregation has not been reported to be a problem with other open-loop insulin delivery systems (Mill-Hill and Autosyr-

ringe), probably because they use less concentrated insulin solutions. However, Autosyringe have modified their device so that now U-100 insulin can be utilized. If insulin aggregation becomes a problem in the tubing near the patient's body, the addition of physiological concentrations of NaHCO_3 should be helpful.

Yours sincerely,
M. B. Davidson

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M. B. Davidson, M. D.
Diabetes Program
Cedars-Sinai Medical Center
870 Beverly Boulevard
Los Angeles
California 90048, USA

Diabetologia (1982) 22: 495–496

Aspects of Non-Enzymatic Glycosylation of Serum Proteins in Relation to Different Glucose Loads

Dear Sir,

Svendsen et al. [1] and Bolli et al. [2], respectively, have shown rapid changes in the concentration of total stable and unstable glycosylated haemoglobin A_{1c} (HbA_{1c}) and HbA_1 to be dependent on short-term fluctuations in blood glucose levels. This phenomenon is regarded as representing the formation and dissociation of unstable HbA_1 (aldimine-linked adduct), which is correlated with significant increases or reductions in glycaemic values.

Recently, non-enzymatic glycosylation of total serum proteins has also been shown to be increased in diabetic patients [3–5]. However, it has not been proven whether rapid changes in glucose levels in vivo and in vitro affect the level of glycosylated serum proteins [5]. We have therefore measured glycosylated serum proteins in ten normal subjects (aged 40 ± 10 years; mean $\pm$ SD) and in 12 Type 1 (insulin-dependent) diabetic patients (aged 38 ± 9 years) after an overnight fast during an oral glucose tolerance test and several days later during a continuous 5 h IV infusion of glucose (0.33 g/kg body weight in a bolus + 0.9 g/min in normal subjects and 0.6 g/min in diabetic patients). Glycosylated serum proteins levels were determined by thiobarbituric acid reaction before and after dialysis of serum samples against 0.15 mol/l saline solution (1:350) at 4°C for 18 h (3). In the diabetic patients, the fasting glucose levels were

higher (8.1 ± 3.1 mmol/l) than in the normal subjects (4.3 ± 0.3 mmol/l). Before dialysis, glycosylated serum proteins, expressed as nmol 5-hydroxymethylfurfural (5-HMF)/mg protein, showed values which were in parallel with the glycaemic values observed before and during the glucose loads. After dialysis, glucose was always < 0.2 mmol/l in samples from both groups, while 5-HMF gave values lower than those observed before removal of glucose ($p < 0.001$). The mean fall was proportionate to glycaemic levels ($p < 0.001$). However, in every assay, diabetic patients showed higher 5-HMF values than normal subjects ($p < 0.001$, Table 1). Moreover, after dialysis, 5-HMF levels were not significantly increased after different glucose loads. In normal and diabetic subjects, they ranged between 0.51 and 0.53 and between 0.93 and 0.97 nmol 5-HMF/mg protein, respectively, during the oral glucose tolerance test with similar values during IV glucose infusion.

In agreement with the findings in vitro of Kennedy et al. [4], our observations in vivo indicate that the determination of glycosylated serum protein levels is influenced by free glucose present in the sample. However, as the oxalic acid used in the thiobarbituric acid reaction test induces rearrangement of the Schiff base [6], it cannot be excluded that the decreases in 5-HMF found after dialysis could

Table 1. Mean values of 5-HMF (nmol/mg protein) observed during different glucose loads, before and after dialysis of serum samples

	Oral glucose tolerance test			Intravenous glucose infusion					
	Time (min)			Time (min)					
	0	60	180	0	5	30	120	240	300
<i>Normal subjects</i>									
Blood glucose (mmol/l)	4.30	7.70	5.00	4.30	13.80	10.90	9.70	10.20	9.60
Before dialysis	0.67	1.02	0.77	0.68	1.17	1.10	0.99	1.05	1.03
After dialysis	0.51	0.52	0.51	0.49	0.50	0.48	0.51	0.48	0.46
Mean $\pm$ SEM fall in 5-HMF	0.16 $\pm$ 0.08	0.51 $\pm$ 0.17	0.26 $\pm$ 0.11	0.19 $\pm$ 0.05	0.67 $\pm$ 0.15	0.62 $\pm$ 0.14	0.48 $\pm$ 0.17	0.56 $\pm$ 0.10	0.57 $\pm$ 0.12
<i>p</i>	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
<i>Diabetic subjects</i>									
Blood glucose (mmol/l)	8.10	15.40	18.70	8.60	20.40	19.70	20.10	20.00	20.00
Before dialysis	1.20	1.44	1.62	1.18	1.61	1.62	1.61	1.63	1.60
After dialysis	0.94	0.95	0.93	0.95	0.96	0.94	0.96	0.92	0.94
Mean $\pm$ SEM fall in 5-HMF	0.25 $\pm$ 0.16	0.49 $\pm$ 0.22	0.70 $\pm$ 0.25	0.23 $\pm$ 0.17	0.65 $\pm$ 0.39	0.66 $\pm$ 0.41	0.56 $\pm$ 0.26	0.71 $\pm$ 0.30	0.66 $\pm$ 0.27
<i>p</i>	< 0.001	< 0.001	< 0.001	< 0.005	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001

be partially related to a deglycosylation of the unstable aldiminic adduct, which, as also seen in the synthesis of HbA_{1c} (1), can be formed *in vivo*, thus constituting a reversible step in the non-enzymatic glycosylation of serum proteins. This observation is also in agreement with the results of Yue et al. [5] who studied glycosylated serum proteins *in vitro* and showed that during dialysis, glucose slowly dissociates from proteins: after 24 h, only 72% of glucose remained bound to the proteins.

The demonstration that, after serum dialysis, the 5-HMF assay does not undergo modifications during the various phases of experimental hyperglycaemia, shows that glycosylated serum proteins are not influenced either by transitory hyperglycaemic peaks or by high plasma glucose concentrations lasting for 5 h. Therefore, it can be concluded that glycosylated serum protein levels are correlated with carbohydrate intolerance although, as determined by the thiobarbituric acid reaction test, they reflect the molecular alterations which can occur in serum proteins in response to increases in blood glucose levels lasting for more than 5 h.

Yours sincerely,

G. Gragnoli, I. Tanganelli, A. M. Signorini, P. Tarli and C. Paoli

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Professor G. Gragnoli
Istituto di Clinica Medica Generale e
Terapia Medica
Università di Siena
Piazzetta della Selva 7
53100 Siena, Italy