Glycosylated Haemoglobin Levels in a Colony of Spontaneously Diabetic Rabbits

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Summary. Elevated glycosylated haemoglobin values have been observed in overtly diabetic animals from a colony of spontaneously diabetic rabbits. Chemically diabetic and normal animals did not show elevated levels. Overtly diabetic animals averaged 12.2% glycosylated haemoglobin versus 4.3% for chemically diabetic and 3.9% for normal animals. Increased levels did not correlate with plasma glucose concentration. Some chemically diabetic and normal animals progressed with time to a more severe diabetic classification. Glycosylated haemoglobin levels at the high end of the range of values for normal animals are predictive of this progression especially in certain litters.

Key words: Glycosylated haemoglobin, diabetes, rabbits

Diabetes mellitus occurs in laboratory animals [1] and diabetic animal models have aided the study of the human disease [2–5]. Recent work with a closed colony of New Zealand White (NZW) rabbits has defined a syndrome characterised by a late onset (1 to 3 years) of fasting hyperglycaemia, an absence of obesity, low fasting immunoreactive insulin (IRI) levels, and mild ketonaemia [6, 7], which resembling non-insulin dependent diabetes occurring in non-obese adults. Overtly diabetic rabbits make up 19% of the colony and rabbits with abnormal glucose disposal ($K_g < 1.0$) without fasting hyperglycaemia, 27%. A decreased ability of secretagogues to stimulate IRI release in rabbits with abnormal glucose disposal [7] correlates with a highly significant hypergranulation of β -cells [8]. This data suggests the involvement of a defective insulin release mechanism in the diabetes of this animal model.

It has been postulated that a number of secondary abnormalities of diabetes might be explained by glycosylation reactions due to elevated circulating glucose levels. One reaction that has recently been

studied extensively is the formation of glycosylated haemoglobins. These consist of three minor haemoglobin components comprising 3–5% of total erythrocyte haemoglobin [9]. The present study describes an examination of glycosylated haemoglobin levels in the spontaneously diabetic NZW rabbit colony.

Methods

Animals from a closed colony of New Zealand rabbits maintained at the VA Medical Center were used for the study. Rabbits were singly caged and fed standard laboratory diet (Purina Lab Rabbit Chow, Ralston Purina Co., St. Louis, Missouri) ad libitum without supplement. Classification, treatment and general handling of the animals has been described previously [7, 8]. Animals with fasting glucose concentrations >150 mg/dl were taken as diabetic. The rest were divided on the basis of IV glucose tolerance (see below) into chemically diabetic ($K_g \leq 1.0$) and normal ($K_g > 1.0$).

Blood was drawn into heparinised Vacutainer tubes, (Fisher Scientific, Springfield, New Jersey), centrifuged and the plasma was separated and stored at -20°C . Pelleted red blood cells were washed twice in 0.14 mol/l NaCl then haemolyzed in iced deionised water and toluene. After the removal of toluene the aqueous layer was treated overnight with Developer 6 [9] at 4°C . Haemoglobin values of the haemolysate were determined using Drabkin's solution (Sigma, St. Louis, Missouri) and external and internal standards (Hycel, Houston, Texas).

For the determination of glycosylated haemoglobin, haemolysates were chromatographed on 0.9×25 cm columns of Bio-Rex 70, 200–400 mesh at 25°C by a modification of the method of Trivelli et al. [9]. Glycosylated haemoglobin fractions were eluted with Developer 6, pH 6.9 and the major haemoglobin fraction with 0.3 mol/l phosphate, pH 6.4. Flow rate was controlled with a peristaltic pump (Technicon) (24 ml/h) and 6 min fractions were collected. Inter- and intrassay variation was 4.5% and 6.2% respectively. Blood glucose levels were measured by the Statzyme procedure (Worthington Biochemical Corp., Greenhold, N.J.), IV glucose tolerance tests were conducted as described previously [7]

and K_g calculated as, $K_g = \frac{0.693}{t_{1/2}} \times 100$). Student's t-test was

used to determine the significance of differences between groups. The elution profile of rabbit glycosylated haemoglobins paralleled that of human and mice haemoglobins [9, 11]. In order to decrease operating time, conditions were selected to elute all glycosylated haemoglobin components in a single peak. This technique has been recently evaluated and shown to be valid [12].

Table 1. Glycosylated haemoglobin values in spontaneously diabetic rabbits

Classification	n	% glycosylated Hb	Kg value $\pm$ SEM
Overtly diabetic	5	12.2 $\pm$ 1.2	—
Chemically diabetic	7	4.3 $\pm$ 1.7	0.53 $\pm$ 0.02
Normal	15	3.9 $\pm$ 1.6	1.52 $\pm$ 0.25

Results are expressed as mean $\pm$ SEM

Table 2. Glycosylated haemoglobin values of individual animals from a colony of spontaneous diabetic rabbits

Animal	Initial glycosylated Hb value (%)	Time span (months)	New classification
120	6.9	10	Overtly diabetic
119	3.6	10	Overtly diabetic
126	6.3	4	Chemically diabetic
117	6.1	9	Overtly diabetic
107	6.1	9	Overtly diabetic
123	11.7	6	Chemically diabetic

Results

Animals were chosen at random from the diabetic colony. Table 1 indicates that the overtly diabetic animals had statistically significant elevated glycosylated haemoglobin values ($p < 0.02$) when compared to either the chemically diabetic or normal colony animals. There were no differences in fasting plasma glucose concentrations between normal and chemically diabetic animals (90–120 mg/dl). Overtly diabetic animals had elevated fasting glucose concentrations (160–500 mg/dl) but there was no correlation with glycosylated haemoglobin levels.

Table 2 lists some animals whose initial Kg values classified them as normal or chemically diabetic but who when tested at intervals of 3–5 weeks, were reclassified as either chemically or overtly diabetic within 4–10 months. With one exception these animals showed the highest glycosylated haemoglobin values of their initial classification group. Two of these animals were from the same litter. In addition, all animals in two other litters have been reclassified from normal to either chemically or overtly diabetic.

Discussion

Overtly diabetic rabbits had significantly elevated levels of glycosylated haemoglobin compared to normal animals. Animals with impaired IV glucose tolerance values ($Kg \leq 1.0$) did not exhibit significant changes in glycosylated haemoglobin levels relative to normal colony animals but some of these chemically diabetic animals developed overt diabetic symp-

toms including elevated glycosylated haemoglobin values. Animals in the normal classification ($Kg \geq 1.0$) especially those whose glycosylated haemoglobin values were high relative to the group average consistently developed more severe diabetes and this trend was consistent within certain litters. Our results indicate that elevated glycosylated haemoglobin levels in colony animals with Kg values > 1.0 can be predictive of chemical or overt diabetes, and further substantiates this unique colony as a model for maturity-onset insulin-dependent diabetes.

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