23 Bengochea, L., Ouvina, G., Sozzani, O., and Lemberg, A., Biochem. biophys. Res. Commun. *144* (1987) 980.
24 Hutterer, F., Denk, H., Bacchin, P. G., Schenkman, J. B., Schaffner, F., and Popper, H., Life Sci. *9* (1970) 977.
25 James, J. L., Moody, D. E., Chan, C. H., and Smuckler, E. A., Biochem. J. *206*, (1982) 203.
26 James, J. L., Friend, D. S., MacDonald, J. R., and Smuckler, E. A., Lab. Invest. *54* (1986) 268.
27 Utsumi, H., Murayama, J. I., and Hamada, A., Biochem. Pharmac. *34* (1985) 57.
28 Reichen, J., Egger, B., Ohara, N., Zeltner, T. B., Zysset, T., and Zimmermann, A., J. clin. Invest. *82* (1988) 2069.

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Preferential excretion of glycated albumin in C57BL-Ks-J mice: Effects of diabetes

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Abstract. Urinary excretion of glycated albumin was quantitated in genetically hyperglycemic mice (C57BL-Ks-J, db/db mice), a model for non-insulin-dependent diabetes mellitus, and compared with their non-diabetic littermates. The data indicated a preferential excretion of glycated albumin in non-diabetic mice. This phenomenon of 'editing' of glycated albumin is decreased significantly in diabetic mice. Quantitative measurements of overall excretion of glycated albumin suggested that the loss of editing in diabetic mice is due to the dilution of glycated albumin by the unmodified albumin which is excreted in large amounts in diabetic mice. Therefore, the loss of editing observed in this model resembled the one we characterized in insulin-dependent diabetic humans and a streptozotocin-diabetic rat model[3].

Key words. C57BL-Ks-J mice; db/db mice; glycated albumin; urinary excretion.

A growing body of experimental evidence suggests that the mammalian nephron selectively excretes glycated albumin into urine. This manifests as a marked increase in the percent of glycated urinary albumin which can reach levels 16 times higher than that of plasma glycated albumin in healthy humans[1-3]. This phenomenon of editing, which is the ratio of percent glycated albumin in urine to glycated albumin in plasma, is lost suddenly in diabetic humans and in streptozotocin-diabetic rats[1,3]. In contrast to the abrupt loss of editing in diabetes, we have also observed a gradual reduction in editing of glycated albumin as a function of age[3] and postulated two different models for this phenomenon associated with diabetes and aging. In diabetes, the attenuation in editing is due to the dilution of glycated albumin by the unmodified albumin, which is excreted in large amounts. On the other hand, in aging nephron, the reduction in editing is the result of decreased filtration of more negatively charged glycated albumin[3,4] into the urine.

The diabetic mutant mouse (C57BL-Ks-J strain, db/db) is widely used as a model for non-insulin-dependent diabetes mellitus, and this mouse has been shown to manifest symptoms like those in adult onset diabetes in man, with obesity and hyperinsulinemia being characteristic features[5]. While it has been shown that diabetic mice develop microvascular complications associated with diabetes[5], the ability of the nephron to selectively discriminate glycated from unmodified proteins has not been studied. The present study was undertaken to see whether the loss in editing observed in streptozotocin-diabetic rats is also manifested in these diabetic mice and, also, to evaluate the possible mechanism(s) underlying such a reduction.

Methods

Diabetic mice (11–12 weeks old) and their non-diabetic littermates belonging to C57BL-Ks-J strain were obtained from the Jackson Laboratory, Bar Harbor, ME. Animals were housed in a light cycled room and cared for in accordance with National Institutes of Health and Institutional Animal Care and Use Committee Guidelines. Degree of hyperglycemia was monitored by quantitating glycated albumin and glycated hemoglobin (HbA1C) as described below.

Collection and processing of blood and urine from diabetic and non-diabetic mice. Blood was collected into heparinized tubes by cardiac puncture. Twenty-four-hour urine collections were done using metabolic cages (Nalgene, Rochester, NY). Two groups of diabetic and two of non-diabetic mice (consisting of four mice per cage) were used in the present study. This grouping of animals was necessary to obtain sufficient volumes of urine for biochemical analyses. Urine was collected on ice in containers containing sodium azide to prevent growth and the total volumes of urine in 24 h measured. Known volumes of urine (2–3 ml) were dialyzed against distilled water to remove salts and other low molecular weight compounds and lyophilized.

Separation and quantitation of glycated albumin. This was carried out essentially according to the procedure described by us[3] with minor modifications. Lyophilized urines were reconstituted in column buffer containing 250 mM ammonium acetate, pH 8.0, containing 50 mM $MgCl_2$ and 0.02% sodium azide $MgCl_2$. Heparinized blood was centrifuged and the plasma was extensively dialyzed against distilled water (at 4 °C). Urine and plasma samples were applied to Glycogel-B column (Pierce, Rockford, IL). Unmodified (non-glycated) proteins were washed off from the column with the column buffer. Glycated (bound) proteins were eluted from the column with 50 mM Tris-HCl buffer, pH 8.5, containing 200 mM sorbitol for those albumin samples assayed by radioimmunoassay[6]. Alternatively, glycated albumin was eluted with 200 mM citrate buffer (pH 4.5) for assay with bromocresol green[7]. This procedure recovered >99% of the protein applied to the column. Urinary albumin was quantitated by radioimmunoassay[6] using rabbit anti-human albumin and goat anti-γ-globulin antisera (Boehringer Mannheim Diagnostics Inc., Houston, TX). Using [^{125}I]human serum albumin as standard, the immunoassay yielded values that were linear in the range of 12.5 to 250 ng. Plasma albumin and HbA1C were spectrophotometrically measured at 630 nm and 412 nm respectively[7,8].

Statistical analysis. Significance of differences between the control and the experimental groups was assessed by the Student's t-test[9].

Results

Diabetic mice, used in the present study, were characterized by their body weights and HbA1C (a long-term integrator of plasma glucose). The data in table 1 show that the body weights of diabetic mice were two times those of non-diabetic mice. Their HbA1C values were 2.4 times higher than their non-diabetic counterparts suggesting substantial increase in plasma glucose in diabetic animals (table 1).

Relative urinary excretion of glycated albumins was quantitated in non-diabetic and diabetic mice and the data are given in table 2. The data showed that by 11 weeks of age, the percent glycated albumin in plasma was significantly higher in diabetic mice as compared with non-diabetic mice. In non-diabetic mice, glycated albumin was preferentially excreted into urine by the nephron, i.e., it was significantly higher in urine as compared with plasma in the same group of animals. Hence, the editing ratio was 12.29% in non-diabetic mice. In diabetic mice, we observed a striking reduction in this editing ratio and the ratio was 11 times higher in non-diabetic mice as compared with diabetic mice. This reduction in editing ratio of glycated albumin in diabetic mice may be due to an increase in plasma glycated albumin as well as decrease in the percent glycated albumin in urine (table 2). We observed significantly higher rates of diuresis as well as urinary protein excretion in diabetic animals suggesting a substantial increment in the glomerular filtration rates. The overall (24 h) excretion of glycated albumin was found to be 916 μg in diabetic mice, in contrast to only 84 μg in non-diabetic mice.

Table 1. Population characterization of non-diabetic and diabetic mice

Type of mice	Body weights (g)	HbA1C (%)
Non-diabetic	26.25 ± 2.44	3.43 ± 1.12
Diabetic	53.36 ± 2.41*	8.33 ± 0.89*

Data are mean ± SEM of six mice in each group. *$p < 0.01$.

Table 2. Renal discrimination of glycated albumin in non-diabetic and diabetic mice

Study parameters	Type of mice Non-diabetic	Diabetic
Plasma glycated albumin (%)	0.95 ± 0.10	3.00 ± 0.21
Urinary glycated albumin (%)	12.25 ± 0.59	3.35 ± 0.30
Editing ratio	12.29 ± 0.62	1.11 ± 0.09
24 h Urine volume (ml)	3.35 ± 0.58	14.39 ± 0.60
24 h Urinary protein (mg)	0.69 ± 0.05	27.35 ± 3.41

Experimental details are described in the text. Data are mean ± SEM of two groups each of non-diabetic and diabetic mice, consisting of four mice per group. Values represented in all the study parameters differed significantly between both the groups with a $p < 0.01$.

Discussion

These data demonstrate that the reduction in the editing ratio of glycated albumin observed in diabetic mice is similar to the one manifested by streptozotocin-diabetic rats reported by us recently[3]. This is the first report on the preferential handling of glycated proteins by the nephron in mice species. To explain the observed discriminatory phenomenon for glycated albumin, one must consider two loci, namely the glomerulus and the proximal tubule. In addition, the relative increase in the negative charge of proteins, in consequence of glycation, must also be taken into consideration[3,4,10]. There is unequivocal evidence in the literature to suggest that glomerular basement membrane acts as a barrier for the filtration of proteins and is extensively negatively charged[11] because of its endowment with glycosaminoglycans and also glomerular epithelial polyanion, the podocalyxin[12]. Therefore, under normal conditions less of glycated albumin is filtered because of its (increased) negative charge[3,4] compared with the unmodified albumin. However, it has been shown that the glomerular negative charge is decreased significantly in diabetes[13,14]. Therefore, if there is a loss of negative charge on the filter, more glycated albumin should be filtered into Bowman's space. This is indeed true since we observed a significant increase in the overall excretion of glycated albumin in urine of diabetic mice. This assumption is based on the

hypothesis that the proximal tubule excludes glycated albumin from the reabsorption process. Using a micropuncture technique, we have shown recently that the unmodified albumin, but not the glycated albumin, is reabsorbed by the proximal tubule in an age-independent manner [15, 16]. Therefore, the paradoxical reduction in the editing ratio observed in diabetic mice, even with an increased plasma glycated albumin and its overall increased excretion into urine, may be due to the consequential dilution of the glycated albumin by the unmodified albumin, which is excreted in large amounts in diabetic mice (table 2). We have observed a similar reduction in the editing ratio of glycated albumin under the conditions of increased osmotic diuresis, such as mannitol infusion into rats [3]. Moreover, we were able to reverse the reduction in editing ratio in these animals after cessation of mannitol diuresis or by treating the diabetic animals with insulin [3].

It is important to note that non-enzymatic glycation of proteins results in significant alternations in their function [20] as well as their recognition properties by different cell types. It has been demonstrated by Williams et al. [21] that the glycated albumin is avidly pinocytosed by the capillary endothelial cells as compared with unmodified albumin. Vlassara et al. [22] have observed a preferential uptake of glycated myelin basic proteins by the peritoneal macrophages. On the other hand, we have also found that the pulmonary macrophages [23] and proximal tubular epithelial cells [15, 16] exhibit preference for unmodified albumin more than glycated albumin. These observations indicate that glycation of proteins can change their folding, function, and recognition properties.

We [17] and others [18] have shown recently that genetically hyperglycemic mice, unlike streptozotocin diabetic rat [8] and diabetic humans [19], do not exhibit reductions in the erythrocyte sodium-potassium ATPase activity. Experimental evidence is also presented to suggest a relative lack of polyol pathway in these mice, thereby preventing the oxidative damage to critical thiols of sodium-potassium ATPase [17, 18]. The observed reduction in editing ratio may not be linked to the polyol pathway since, we have failed to reverse or prevent this phenomenon in streptozotocin-diabetic rats with sorbinil, an aldose reductase inhibitor, therapy [3].

In summary, we have demonstrated the phenomenon of preferential handling of glycated albumin by the nephron in C57BL-Ks-J-mice and that it is lost in their diabetic counterparts. Moreover, these data indicate that a similar mechanism may underlie the reduction of editing ratio in insulin and non-insulin dependent diabetes.

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1 Williams, S. K., Devenny, J. J., Pinter, G., and Bitensky, M. W., Microvasc. Res. *3* (1983) 96A.
2 Ghiggeri, G. M., Candiano, G., Delfino, G., Bianchini, F., and Queirolo, C., Kidney. Int. *25* (1984) 565.
3 Kowluru, A., Kowluru, R. A., Bitensky, M. W., Corwin, E. J., Solomon, S. S., and Johnson, J. D., J. exp. Med. *166* (1987) 1259.
4 Harding, J. J., Beswick, H. T., Ajiboye, R., Blakytny, R., and Rixon, K. C., Mech. Aging. Dev. *50* (1989) 7.
5 Coleman, D. L., Diabetes *31* Suppl. 1 (1982) 1.
6 Woo, J. M., Floyd, D. C., Cannon, D. C., and Kahan, B., Clin. Chem. *24* (1978) 1464.
7 Pierce Bioresearch Products Technical Bulletin. Pierce Chemical Co. Rockford, IL (1983).
8 Kowluru, R. A., Bitensky, M. W., Kowluru, A., Dembo, M., Keaton, P. A., and Buican, T., Proc. natl Acad. Sci. (USA) *86* (1989) 3327.
9 Snedecor, G. W., Statistical Methods. Iowa State University Press, Ames (1956).
10 Bitensky, M. W., Kowluru, A., and Kowluru, R. A., in: Maillard Reactions in Aging, Diabetes, and Nutrition, p. 185. Eds J. W. Baynes and V. M. Monneir. Alan R. Liss. Inc., New York 1989.
11 Rennke, H., Cotran, R. S., and Venkatachalam, M. A., J. cell Biol. *67* (1975) 638.
12 Kerjaschki, D., Sharkey, D. J., and Farquahr, M. G., J. cell Biol. *98* (1984) 1591.
13 Westberg, N. G., Diabetes *25* Suppl. 2 (1976) 920.
14 Parthasarathy, N., and Spiro, R. G., Diabetes *31* (1982) 738.
15 Kowluru, A., Bitensky, M. W., and Kowluru, R. A., Diabetes *39* (1990) 29A.
16 Kowluru, A., Kowluru, R. A., Solomon, S. S., and Martinez, L., Life Sciences *50* (1992) 281.
17 Kowluru, R. A., and Kowluru, A., Metabolism *41* (1992) 160.
18 Bianchi, R., Marelli, C., Marini, P., Fabris, M., Triban, C., and Fiori, M. G., Diabetologia *33* (1990) 131.
19 Finnoti, D., and Palatini, P., Diabetologia *29* (1986) 623.
20 Kowluru, R. A., Heidorn, D. B., Edmondson, S. P., Bitensky, M. W., Kowluru, A., Downer, T. W., Whaley, T. W., and Trewhella, J., Biochemistry *28* (1989) 2220.
21 Williams, S. K., Devenny, J. J., and Bitensky, M. W., Proc. natl Acad. Sci. (USA) *78* (1981) 2393.
22 Vlassara, H., Brownlee, M., and Cerami, A., Diabetes *34* (1985) 553.
23 Bitensky, M. W., Kowluru, R. A., Kowluru, A., Valdez, Y. E., Tillery, S. I., and Lehnert, B. E., Proc. endocr. Soc. *106* (1986) 301 (Abstract).

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