Relationship Between Serum 1,5-Anhydroglucitol and Urinary Excretion of *N*-Acetylglucosaminidase and Albumin Determined at Onset of NIDDM With 3-Year Follow-up

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OBJECTIVE — This prospective study was designed to elucidate the relationship between the serum level of 1,5-anhydroglucitol (1,5AG) and the urinary excretion of *N*-acetylglucosaminidase (NAG) and albumin in patients who were in the early stages of diabetes.

RESEARCH DESIGN AND METHODS — A total of 1,062 male nondiabetic subjects with impaired glucose tolerance were monitored for blood glucose level once every 2–3 months, and the values were evaluated. Of these 1,062 subjects, 112 showed a worsening of glycemia during the observation period to the level seen in diabetes. We began to monitor the glycemia and parameters of renal damage in the 112 patients from the onset of diabetes.

RESULTS — The urinary excretion of NAG and albumin were elevated even at the onset of diabetes. The abnormal excretion of NAG and albumin was associated with a change in serum 1,5AG and was quickly reversible when the serum 1,5AG improved. In the 3 years after the onset of diabetes, we obtained at least 18 measurements of one parameter for each patient and calculated the mean. Urinary NAG was found to be significantly correlated with the fasting plasma level of glucose (FPG; $r = 0.512$, $P < 0.0001$), the level of HbA_1 ($r = 0.351$, $P = 0.001$), and the level of 1,5AG ($r = -0.790$, $P < 0.0001$). The urinary excretion of albumin was weakly but significantly correlated with levels of FPG ($r = 0.383$, $P < 0.0001$) and HbA_1 ($r = 0.337$, $P < 0.0001$), but it was more strongly correlated with 1,5AG ($r = -0.632$, $P < 0.0001$). The level of 1,5AG was significantly correlated with FPG ($r = -0.681$, $P < 0.0001$) and HbA_1 ($r = -0.609$, $P < 0.0001$).

CONCLUSIONS — When the renal damage is not severe, the serum level of 1,5AG appeared to be an indicator of the reversible renal damage caused by hyperglycemia, as well as of the severity of the glycemia itself.

1,5-Anhydroglucitol (1,5AG), which was developed in Japan as a new marker for glycemia (1–11), can rapidly detect a slight change in glycemia and is therefore suitable for monitoring the control of glycemia in patients with near-normoglycemia (12). The 1,5AG is excreted into the urine when its concentration in serum exceeds the renal threshold for reabsorption (13–15). The renal reabsorption of 1,5AG is competitively inhibited by glucose (13), which is thought to be the major mechanism for the reduction in 1,5AG in patients with diabetes.

Because 1,5AG is reabsorbed by the renal tubule, its serum level can be influenced by severe renal failure (16,17). In general, advanced renal glomerular disease, such as uremia, can reduce the serum level of 1,5AG in the absence of hyperglycemia (3,16,17). The serum level of 1,5AG is not affected by nephrosis (3). However, Tetsuo et al. (18) reported that the serum level of 1,5AG was decreased during normal pregnancy, presumably due to mild renal tubular changes. That the urinary excretion of *N*-acetylglucosaminidase (NAG) shows a negative correlation with serum concentrations of 1,5AG in patients receiving prolonged hyperalimentation (19) suggests that tubular damage can reduce the serum level of 1,5AG. Severini et al. (20) reported that an isoenzyme of NAG was significantly correlated with the status of glycemia. In a report of the U.K. Prospective Diabetes Study Group (21), the decrease in urinary albumin and NAG excretion was associated with a reduction in the fasting plasma glucose (FPG) level in response to dietary treatment. Thus, we postulated that 1,5AG may be a sensitive index of renal damage induced by diabetes as well as of hyperglycemia.

This prospective study was conducted in nondiabetic subjects with impaired glucose tolerance to try to evaluate the relationship between serum 1,5AG and the urinary excretion of NAG and albumin in the early stages of diabetes. To evaluate the direct influence of glycemia, participants were limited to middle-aged Japanese businessmen who did not have other diseases or disorders (22) that would adversely affect renal function.

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Abbreviations: 1,5AG, 1,5-anhydroglucitol; CV, coefficient of variation; FPG, fasting plasma glucose; IGT, impaired glucose tolerance; NAG, *N*-acetylglucosaminidase; OGTT, oral glucose tolerance test; WHO, World Health Organization.

RESEARCH DESIGN AND METHODS

Subjects and protocol

Participants were selected from Japanese male businessmen between the ages of 40

Table 1—*Laboratory findings in the 112 subjects monitored for 3 years*

	Onset	End point
BMI (kg/m²)	23.8 ± 0.2	23.5 ± 0.2
Blood pressure		
Systolic (mmHg)	122 ± 1	122 ± 1
Diastolic (mmHg)	79 ± 1	79 ± 1
Total protein (g/l)	74.0 ± 0.4	73.1 ± 0.4
Serum creatinine (μmol/l)	84.0 ± 1.0	83.1 ± 1.2
Uric acid (mmol/l)	0.31 ± 0.01	0.32 ± 0.01
Total cholesterol (mmol/l)	5.25 ± 0.07	5.25 ± 0.06
HDL cholesterol (mmol/l)	1.32 ± 0.03	1.32 ± 0.03
Triglyceride (mmol/l)	1.38 ± 0.06	1.40 ± 0.05
Hemoglobin (g/dl)	15.5 ± 0.1	15.6 ± 0.1

Data are means ± SE. No statistically significant difference was found between data obtained at onset and the end point. End point refers to 3 years after the onset of disease. Onset was the time at which the patients' glucose levels rose to the level used in diagnosing diabetes.

and 50 years who had received routine medical checkups at our hospital in the past 5 or more consecutive years. A total of 1,062 men exhibited a pattern of impaired glucose tolerance (IGT) in the 75-g oral glucose tolerance test (OGTT) during that period. IGT was diagnosed according to the World Health Organization (WHO) criteria. Excluded from study were subjects with a history of coronary heart disease (defined as ischemic symptoms with simultaneous ischemic change in the electrocardiogram or a previous history of myocardial infarction), nephropathy (serum creatinine ≥120 μmol/l), urolithiasis, chronic urinary tract infection, hypertension (systolic blood pressure ≥140 or diastolic blood pressure ≥90 mmHg), hyperuricemia (serum uric acid ≥0.42mmol/l), and dyslipidemia (total cholesterol ≥6.47, HDL cholesterol ≤1.03, or triglyceride ≥2.26 mmol/l). Also excluded from evaluation were heavy smokers (≥10 cigarettes/day), athletes, obese subjects (BMI ≥28 kg/m²), or patients who were previously diagnosed with diabetes (WHO criteria) .

The status of glycemia was monitored once every 2–3 months for 3 years in the 1,062 nondiabetic subjects with IGT. During that period, 112 of 1,062 men developed diabetes, which was diagnosed in accordance with WHO criteria by FPG or OGTT. A total of 95 of the 112 men were diagnosed by the 2-h plasma glucose value by OGTT according to WHO criteria. At the time of diagnosis, the 112 men with NIDDM were aged 46.2 ± 0.4 years (range 41–50 years); the mean duration of IGT was 5.1 ± 0.2 years (range 2–13 years). FPG, HbA_1, serum 1,5AG, urinary NAG excretion, and urinary albumin excretion were monitored every 1–2 months for the next 3 years. Thus, the starting point of this study was the month at which each patient was diagnosed with diabetes. Treatment in 31 patients involved the administration of a sulfonylurea, and 12 patients were changed from a sulfonylurea to diet alone. None of the patients received insulin during the study. All patients received advice regarding diet and/or exercise.

Urinary NAG and albumin in the first morning urine samples and in the 24-h collection samples of the same day were measured in 48 samples obtained from 27 nondiabetic volunteers (mean age 45 years) to assess the accuracy of the first morning urine samples. An age-matched normal range for urinary albumin and NAG excretion were provided by the same samples obtained from the same volunteers.

The patients came to the outpatient clinic of our hospital after an overnight fast during which they were permitted to drink water. Samples of urine and blood were collected. After the diagnosis of diabetes was confirmed, patients were instructed to follow a diet that was high in carbohydrate, low in saturated fat, and moderately high in fiber with a reduced energy content. In addition, the patients met with a nurse-educator and a dietitian several times a year for 3 years. Blood pressure was measured at each visit. When a symptomatic urinary tract infection (>10^5 bacteria/ml) was present, the evaluation of NAG and albumin excretion was postponed until the infection had been effectively treated. All subjects gave their informed consent for participation. The study protocol was approved by the ethics committee of our institution.

Measurements

Blood pressure was measured with the patient in the sitting position after a 5-min rest, with the forearm supported and semiflexed and with the palm facing upward. Hypertension was diagnosed if the systolic blood pressure was ≥140 mmHg or diastolic blood pressure was ≥90 mmHg, or if the patient was receiving antihypertensive therapy. Glucose concentration was measured from a venous blood sample by the glucose oxidase method. HbA_1 (normal range: 4.8–7.5%) was measured by high-performance liquid chromatography (HLC-723 GBH; Tosoh, Tokyo) with an interassay coefficient of variation (CV) of 2.5%. In this study, the collection of urine and its timing varied according to the schedules of these businessmen. We therefore used the albumin:creatinine ratio (mg albumin:g creatinine) of the first morning urine sample (23) as an indicator of albuminuria. Sodium benzoate 0.5 g was added to the early morning urine sample as a preservative. Urinary volume was recorded at that time. Urinary albumin concentration (normal range: <13.6 mg/g creatinine) was determined by radioimmunoassay (Diagnostic, Los Angeles, CA). The lower limit of the assay was 2 mg/l albumin; the interassay CVs were <10%. The urinary concentration of creatinine was measured by a modified method of Jaffe's reaction on an Astra-7 automated system (Beckman Instruments, Brea, CA). Urinary NAG (normal range: <5.0 U/l) was determined with a kit using the m-cresol purple method (Shionogi Pharmaceutical, Osaka, Japan). All three assays were performed on the same urine sample. Interassay CVs were <10% for urinary NAG and <5% for creatinine. The serum concentration of 1,5AG (normal range: 14.0–39.0 μg/ml) was determined with an autoanalyzer system (Automatic Clinical Analyzer, Model 7150, Hitachi, Tokyo) (24) using a modified column enzymatic test (25). The interassay CV was 4.5%, and the intra-assay CV was 1.0%.

Statistical analysis

Date are presented as means ± SE. Differences between groups were compared by Student's *t* test for unpaired data or analysis of variance (ANOVA). Differences between groups were estimated by Scheffe's test for single subgroups. Correlation coefficients were determined by linear regres-

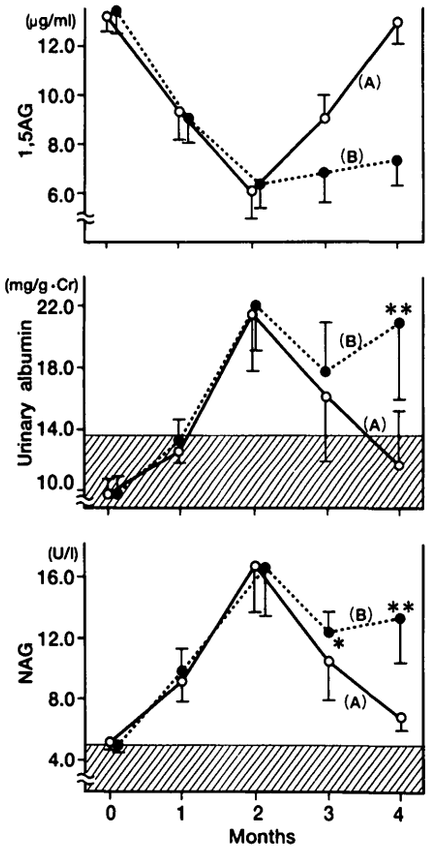

Figure 1—*Serial changes in the urinary excretion of albumin and NAG 2 months after glycemia had increased to the level diagnostic of diabetes. There was a rapid response to the improvement of the 1,5AG. Group A consisted of 68 patients whose glycemia (indicated by 1,5AG) responded to dietary modification starting 2 months later. Group B consisted of 54 patients whose glycemia responded poorly. *P < 0.05, **P < 0.01 vs. group A. Horizontal lines indicate the boundaries of normal ranges.*

sion. Multiple regression analysis was used to assess the joint effects of variables associated with the serum level of 1,5AG. Statistical analyses were performed using the JMP 2.0 statistical software program (SAS, Tokyo, Japan). A level of $P < 0.05$ was accepted as statistically significant.

RESULTS — In the 27 nondiabetic volunteers, the mean urinary NAG in the first morning urine sample was 2.8 ± 0.2 U/l (range 0.6–6.4 U/l) and that in the 24-h urine sample was 6.1 ± 0.3 U/day (range 0.9–18.3 U/day). The mean urinary albumin:creatinine ratio in the first morning urine sample was 7.4 ± 0.4 mg/g creatinine (range 2.1–24.5 mg/g creatinine). In the 27 nondiabetic volunteers, the mean albumin in the 24-h collection sample was 11.7 ± 0.6 mg/day (range 2.0–32.7 mg/day). Both NAG ($r = 0.88$, $P < 0.001$) and the albumin:creatinine ratio ($r = 0.91$, $P < 0.001$) showed strong correlations with each 24-h excretion value. Urinary NAG and albumin were not correlated with FPG and did not correlate significantly with HbA_1 ($r = 0.17$ and $r = 0.15$, respectively) or 1,5AG ($r = 0.14$ and $r = 0.15$, respectively).

In the 112 patients, monitoring of glycemia and of renal abnormalities was begun the month after the diagnosis of diabetes and continued for 3 years. The patients were encouraged to maintain their diet and exercise regimens and to take an oral antidiabetic agent as indicated. During the study period, nearly all of the 112 patients maintained a mean HbA_1 showing good to fair glycemic control (8.1 ± 0.1%); none had an HbA_1 >10%. Retinopathy and/or other severe complications related to diabetes were not observed in any patient. Table 1 summarizes the changes in the clinical laboratory findings over the 3-year period. No significant change in primary clinical data, such as body weight or blood pressure, was noted during this period. At the time of onset, glycemic deterioration was so mild, and was detected so early, that none of the patients reported any diabetic complaints, such as thirst or nocturia, and none exhibited weight loss.

We monitored the parameters for glycemia and for renal damage monthly for 4 months after disease onset. Strict dietary control and exercise therapy were started 2 months after onset, because all of the abnormalities present at onset had been confirmed by this time. The patients were divided into two groups according to the response of their 1,5AG value to therapy. We compared their parameters of renal damage (Fig. 1). Group A consisted of 68 patients whose 1,5AG level increased more than 3.0 μg/ml within 2 months of initiating treatment; group B contained the remaining patients. The abnormal excretion of NAG and albumin observed in these subjects in the early stage of NIDDM was associated with a change in 1,5AG. No significant differences were observed between the two groups in HbA_1, BMI, or blood pressure in the 2nd month.

In each patient, at least 18 values were obtained for each parameter over the 3-year period. Mean values were calculated. The status of urinary NAG and albumin excretion in patients with a low to high serum concentration of 1,5AG during this period are compared in Tables 2 and 3. As shown in Table 2, in each comparison of the two subgroups, the level of NAG excretion differed significantly between the groups.

Table 2—*Comparison of the amount of urinary NAG excreted with the serum level of 1,5AG over a 3-year period*

1,5AG (μg/ml)			NAG (U/l)				
Range	Mean	*n*	Onset	End point	*P* value	Mean	*P* value
≥10.0	11.8 ± 0.2	25	4.7 ± 0.2	7.6 ± 0.6	<0.001	7.7 ± 0.5	0.003
6.0–9.9	8.1 ± 0.2	47	5.1 ± 0.1	12.4 ± 2.2	<0.001	9.7 ± 0.4	0.002
≤5.9	4.2 ± 0.1	40	4.9 ± 0.2	23.2 ± 3.6		14.5 ± 0.3	

Data are means ± SE. End point refers to 3 years after the onset of disease. Data at the time of onset were obtained at diagnosis, or month 0, as described in Fig. 1. Means are the average of 18 determinations of each parameter (or marker) during a 3-year period for each patient.

Table 3—*Comparison of the amount of urinary albumin excreted with the serum level of 1,5AG over a 3-year period*

1,5AG (μg/ml)			Albumin (mg/g creatinine)				
Range	Mean	*n*	Onset	End point	*P* value	Mean	*P* value
≥10.0	11.8 ± 0.2	25	9.6 ± 0.7	8.6 ± 3.2	0.001	8.3 ± 0.5	0.003
6.0–9.9	8.1 ± 0.2	47	10.0 ± 0.8	16.8 ± 3.7	<0.001	10.9 ± 0.7	0.002
≤5.9	4.2 ± 0.1	40	9.9 ± 0.9	62.3 ± 6.2		29.9 ± 2.7	

Data are means ± SE. End point refers to 3 years after the onset of disease. Data at the time of onset were obtained at diagnosis, or month 0, as described in Fig. 1. Means are the average of 18 determinations of each parameter (or marker) during a 3-year period for each patient.

Table 4—*Urinary excretion of NAG and albumin in patients with low versus high mean HbA_1 or FPG level over a 3-year period*

	Range	Mean	*n*	NAG (U/l)	*P* value	Albumin (mg/g creatinine)	*P* value
HbA_1 (%)							
	≥8.0	8.5 ± 0.1	74	11.6 ± 0.4	0.003	19.9 ± 1.9	0.001
	≤7.9	7.5 ± 0.1	38	9.7 ± 0.6		11.6 ± 1.0	
FPG (mmol/l)							
	≥7.8	8.7 ± 0.1	70	14.0 ± 0.5	<0.001	22.5 ± 2.2	0.001
	≤7.7	6.8 ± 0.1	42	8.7 ± 0.6		9.8 ± 0.8	

Data are means ± SE. Means are the average of 18 determinations of each parameter (or marker) during a 3-year period for each patient.

Table 5—*Correlations between two parameters of renal damage and three markers for glycemia in 112 patients*

	Indicator for renal damage	
Marker for glycemia	NAG	Albumin
FPG	0.512*	0.383*
HbA_1	0.351†	0.337*
1,5AG	−0.790*	−0.632*

Data are derived from 112 patients. Pearson correlation analysis was performed based on the mean value of 18 determinations of each parameter (or marker) during a 3-year period for each patient. *$P < 0.0001$; †$P = 0.001$.

Similar results were observed in the comparison of the amount of urinary albumin excreted with the level of 1,5AG (Table 3). When these patients were classified into those with a mean HbA_1 above or below 8.0%, both the mean NAG level and the amount of albumin excretion were also significantly higher for those with a mean HbA_1 above 8.0% for the 3-year period (Table 4). Similar results were obtained from data corresponding to subdivision by FPG level (Table 4). The correlation coefficients for each parameter are compared in Table 5. NAG and albumin were each correlated with the severity of glycemia, as assessed by FPG, HbA_1, and 1,5AG. Although the mean HbA_1 value was significantly correlated with the mean values for NAG and albumin, the *r* values for correlations were much lower than those indicated by the mean values for FPG and 1,5AG. The relationships between 1,5AG and HbA_1 and NAG are illustrated in the scattergram (Fig. 2). In multivariate analyses (Table 6), both glycemia (FPG) and renal damage (NAG) were found to be associated with the level of 1,5AG. Neither the excretion of NAG nor the level of albumin was influenced by type of medication, diet, or hypoglycemic agent administered (data not shown). Statistically significant correlations were demonstrated between the following: urinary NAG value and the amount of albumin excreted ($r = 0.556$, $P < 0.0001$); the level of 1,5AG and FPG ($r = -0.681$, $P < 0.0001$); the level of 1,5AG and HbA_1 ($r = -0.609$, $P < 0.0001$), and the level of HbA_1 and FPG ($r = 0.633$, $P < 0.001$).

CONCLUSIONS — The present study showed that the urinary excretion of albumin and NAG was associated with the serum concentration of 1,5AG in the initial stage of NIDDM. In particular, NAG excretion was strongly correlated with that of 1,5AG. The site of 1,5AG reabsorption in the kidney is thought to be downstream of the main glucose transport system (26,27). Although the serum concentration of 1,5AG seems to show a nonspecific reduction in patients with renal tubular damage, its level is not usually decreased in those with tubular damage related to hypertension or hyperuricemia (3). However, an abnormal reduction in serum 1,5AG has been observed in nondiabetic patients who were administered hyperalimentation for long periods (19). The urinary excretion of 1,5AG was closely correlated ($r = 0.792$) with that of NAG, but not with the serum creatinine level or with the urinary excretion of microalbumin or of urinary β_2-microglobulin. These results indicate that a specific type of renal tubular damage

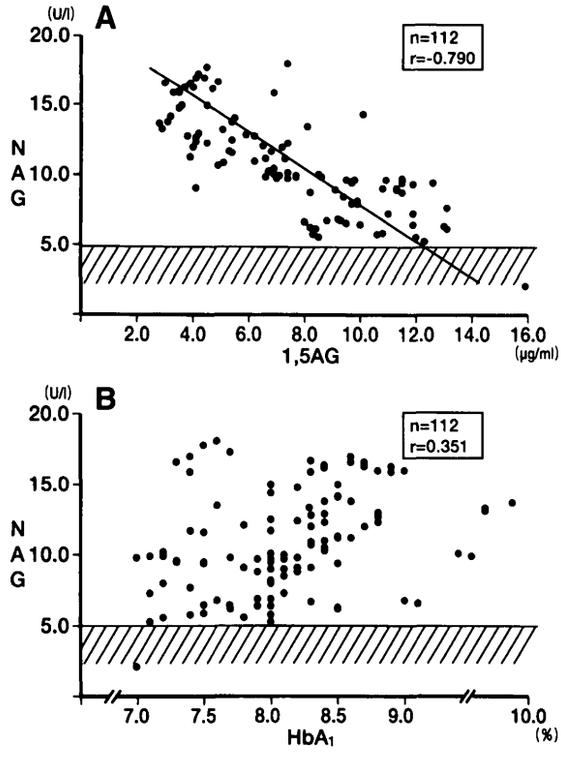

Figure 2—*Comparison of mean serum levels of 1,5AG and of HbA_1 with the mean urinary level of NAG. Data were obtained during monitoring for 3 years after the initial deterioration of the subject's glycemia. Each point is the mean of 18 measurements obtained in 112 patients over the 3-year period.* A: *relationship between 1,5AG and NAG;* r = *0.790,* P < *0.0001.* B: *relationship between HbA_1 and NAG;* r = *0.351,* P = *0.001. Horizontal lines indicate the boundary of the normal range of urinary NAG excretion.*

Table 6—*Multivariate associations of 1,5AG in patients in the early stage of NIDDM*

Independent variable	Regression coefficient	SE	*t* value	*P* value
Intercept	24.119	1.9840	12.157	0.0001
NAG	−0.4624	0.0498	−9.288	0.0001
FPG	−0.0403	0.0113	−3.557	0.0006
HbA_1	−0.6868	0.3006	−2.285	0.0243
Albumin	−0.0244	0.0131	−1.871	0.0641

$R^2 = 0.7683$; $n = 112$.

causes a reduction in 1,5AG. The damage may involve the site at which the 1,5AG transport system is distributed.

We initiated the monitoring of patients with NIDDM as close to the diagnosis (onset) of diabetes as possible. We studied only adult men and excluded any subjects with conditions that are reported (22,28) to influence renal function, such as the elderly and those with hypertension, hyperlipemia, or hyperuricemia. Under these strict conditions, we confirmed that the abnormal urinary excretion of albumin and NAG began at the point at which hyperglycemia reached the level required for the diagnosis of diabetes. We observed a positive correlation between the parameters of renal damage, especially NAG, and 1,5AG. These changes were quickly reversible, suggesting that the renal damage was not severe. A positive correlation between the parameters for renal damage and FPG was also observed. A strong correlation was demonstrated between 1,5AG and FPG. Thus, the renal damage observed was dependent on glycemia. The U.K. Prospective Diabetes Study Group observed that the excretion of urinary albumin and NAG decreased in response to 3 months of dietary treatment, which lowered the FPG (21). In addition, the degree of glycemia had a greater effect on NAG than on albumin in cases of newly diagnosed NIDDM, which is consistent with the present results. In contrast, a weak correlation between parameters of renal damage and HbA_1 was observed. We emphasize that 1,5AG can detect a change in glycemia without delay, whereas the change in HbA_{1c} is delayed for about 1 month (12). Ellis et al. (29) found that urinary NAG significantly correlated with urinary glucose during 10 days at a summer camp for diabetic children. Brouhard et al. (30) reported that HbA_{1c} was not correlated with the urinary excretion of NAG, in that NAG reflects the change in blood glucose over a shorter period of time than does HbA_{1c}. They argued that NAG may vary within a 24-h period, reflecting blood glucose levels. Isoforms of NAG have been identified (31,32). Severini et al. (20) reported that the B isoenzyme of NAG closely reflects metabolic control and can be used as an even earlier and more sensitive indicator of the status of the diabetes than HbA_{1c}. Further studies on the distribution of the NAG isoenzyme in the renal tubule may help to identify the site of damage in the early stages of diabetes.

Thus, the specific type of renal tubular dysfunction caused by hyperglycemia may lead to a reduction in 1,5AG and an increase in urinary NAG in patients with diabetes.

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