

# Plasma 1,5-Anhydro-D-Glucitol as New Clinical Marker of Glycemic Control in NIDDM Patients

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To elucidate the value of using plasma 1,5-anhydro-D-glucitol (AG) as a marker of glycemic control in diabetic patients, the relationship between the plasma concentration of AG and glucosuria was examined in 152 patients with non-insulin-dependent diabetes mellitus (NIDDM). After recovery from the deterioration of glycemic control in NIDDM patients had started, AG began to increase day by day. The recovery of plasma AG showed a constant linear increase curve when excellent glycemic control was attained. The ordinary daily recovery rate of plasma AG was estimated to be 0.3  $\mu\text{g}/\text{ml}$ , which was independent of body weight, sex, age, the difference in treatment, the duration of diabetes, or the level of plasma AG among NIDDM patients. This rate decreased according to the increase in urinary glucose. When we calculated the decrease rate of plasma AG ( $\Delta\text{AG}$ ), assuming 0.3  $\mu\text{g}/\text{day}$  to be the maximum increase rate in a day, we found a high correlation between  $\Delta\text{AG}$  and urinary glucose at almost all AG levels except the normal range and observed that plasma AG (A) times urinary glucose (G) was relatively constant. The formula  $A \times G = 16$  is a simple equation for rough estimation of urinary glucose from the plasma AG concentration in a stable glycemic-controlled NIDDM patient, and we call it the *A · G index*. The plasma AG also correlated significantly with fasting plasma glucose ( $r = -.810$ ) and glycosylated hemoglobin ( $r = -.856$ ) in the same stable glycemic-controlled NIDDM patients. Based on these observations, we propose that plasma AG can serve as a new marker that may provide sensitive and analytical information about glycemic control. *Diabetes* 38:723–29, 1989

**D**ecreased plasma 1,5-anhydro-D-glucitol (AG) has been reported in experimental diabetic animals (1) and diabetic humans (2–4). This reduction has been sensitively and specifically (5) demonstrated in diabetes mellitus. However, the mechanism underlying plasma AG reduction in a diabetic condition has remained unknown. Recently, we reported that the reduction of plasma

AG in diabetic rats might be associated with the accelerated urinary excretion of AG, which is coincident with the excretion of glucose (6). Although AG has a similar structure to glucose, its metabolism is suggested to be low in rats (1,7) and humans (8). However, it was also suggested that the mechanism of AG uptake into the brush border is at least partly common to that of glucose uptake in rat kidney. These facts led to the hypothesis that the alteration of plasma AG is dominantly affected by the amount of urinary glucose. Thus, the measurement of plasma AG possibly provides information about urinary glucose in humans too. Therefore, in this study, we examined the relationship between the plasma AG concentration and urinary glucose in non-insulin-dependent diabetes mellitus (NIDDM) patients by accurate measurement of AG.

## MATERIALS AND METHODS

**Subjects and protocol.** The plasma AG concentration was examined in 156 NIDDM patients (80 men, 76 women), including 1 with gestational diabetes mellitus. Some of these patients had various complications arising from diabetes mellitus, but patients with other diseases or severe diabetic nephropathy (plasma creatinine level  $>0.2$  mM) were excluded. Patients with urinary infectious diseases or taking special kinds of drugs that may have affected measurement of urinary glucose were also excluded. The mean age was 56 yr (range 27–82 yr), and the mean duration of diabetes was 8.3 yr (range 3 mo–26 yr). Subjects were classified as

1,5-Anhydro-D-glucitol	1 $\mu\text{M}$ = 0.18 $\mu\text{g}/\text{ml}$	Insulin	1 pM = 0.139 $\mu\text{U}/\text{ml}$
Glucose	1 mM = 18 mg/dl		

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NIDDM patients according to National Diabetes Data Group criteria (9). The discrimination of NIDDM was confirmed by analysis of the C-peptide response to glucagon as described previously (10). The patients composed four groups. First, 18 inpatients (all of whom later became outpatients) with poor glycemic control were monitored frequently for serum glucose, AG, glycosylated hemoglobin (HbA<sub>1c</sub>), and urinary glucose for 6–12 mo after starting various treatments. These patients were of various ages and body weights and were matched according to their sex. After a longitudinal study was started, the 18 patients were further divided into two subgroups. One subgroup showed remarkable improvement in glycemic control, with normalization of the plasma AG level (*n* = 14; Table 1), and the other showed continuing relatively poor glycemic control (*n* = 4).

Longitudinal close examination of the correlation between plasma AG and urinary glucose was carried out for the second group, 6 inpatients (3 men, 3 women; 36–68 yr old). In these patients, plasma AG and daily urinary glucose were examined every day for 1–2 mo. In the third group, composed of 85 inpatients (5 treated with insulin plus sulfonylureas, 76 with a diet and sulfonylureas, and 4 with diet alone) with various plasma AG levels, the plasma AG and daily urinary glucose were monitored daily for 3–7 days. The degree of plasma AG reduction and the amount of daily urinary glucose during the AG change were compared. The fourth group comprised 47 outpatients (40 treated with diet and sulfonylureas, 7 with diet alone) selected from the patients whose glycemic control was stable for at least the last 12 wk of study; i.e., the variance of both the HbA<sub>1c</sub> and AG levels was ±10% during that period. The daily urinary glucose, fasting plasma glucose (FPG), HbA<sub>1c</sub>, and plasma AG

levels were determined at 4-wk intervals and are presented as the averages of the last three determinations.

**Sampling.** Unless otherwise noted, the subjects fasted for 14 h before samples for plasma glucose, AG, and HbA<sub>1c</sub> were taken. The 24-h urine was collected from each patient, with 5 g sodium benzoate as a preservative, and refrigerated until processed. Usually, the 24-h urine was collected at 0700 and measured. The plasma sample for AG measurement was obtained just after the 24-h urine was collected.

**Assays.** Glucose concentrations were determined with a Dri-Chem 2000 analyzer (Fuji, Tokyo; 11). HbA<sub>1c</sub> was assayed with a column system from Bio-Rad (Richmond, CA). All column assays were carried out in a water bath maintained at 23°C, and quality control was assessed on the basis of variability of the data from fresh normal samples. Standard glucagon tests were performed by the method of Poulsen et al. (10), in which serum C-peptide reactivity was determined in blood obtained 6 min after a 1-mg i.v. glucagon load according to the method described by Hsieh and Ak-anuma (12).

Quantitative glucose evaluation was performed by an aldose 1-epimerase–glucose oxidase method (13). The plasma AG concentration was determined by gas chromatograph–mass spectrometer (GCMS) system (model DX-300, JEOL, Kyoto, Japan); the internal standard was AG-d<sub>6</sub> (14). Plasma (0.2 ml) was added to a fixed amount of an internal standard (10 μl) and mixed with 15 μl of trichloroacetic acid (Sigma, St. Louis, MO). After vortexing, the resultant protein precipitate was removed by centrifugation, and the supernatant (100 μl) was applied to a two-layer column containing, from the bottom, the H form (0.3 ml) of the cation exchanger (AG50W X8) and the OH form (0.5 ml)

TABLE 1  
Clinical characteristics and increase rate of plasma 1,5-anhydro-D-glucitol (AG) from low level in patients with strictly controlled NIDDM

Patient	Age (yr)	Sex	Body weight* (kg)	Duration of diabetes (yr)	Treatment†		Diet (cal/day)	Days	HbA <sub>1c</sub> ‡ (%)	AG (μg · ml <sup>-1</sup> · day <sup>-1</sup> )
					Drug	Dose				
1	31	M	80	12	Glyburide	1.25 mg	1600	19	16.2	0.14
					Glyburide	2.5 mg	1600	48	6.3	0.30
2	39	F	58	Gestational	Diet only		1800	48	9.2, 5.2	0.30
3	41	M	50	5	Diet only		1600	49	11.0, 6.6	0.22
4	46	M	67	0.25	Glyburide	1.25 mg	1600	58	15.5, 6.0	0.29
5	47	F	74	2	Glyburide	2.5 mg	1600	53	13.8, 6.5	0.28
6	50	F	45	14	Insulin	14, 10 U	1400	24	14.5	0.21
					Glyburide	1.25 mg	1200	26	6.1	0.30
7	53	M	66	0.5	Insulin	16, 10 U	1600	28	14.1	0.24
					Glyburide	1.25 mg	1400	21	6.3	0.31
8	55	M	55	4	Insulin	12, 6 U	1200	28	19.8	0.19
					Diet only		1400	56	6.1	0.23
9	56	F	51	6	Glyburide	1.25 mg	1200	20	14.8	0.20
					Glyburide	2.5 mg	1200	28	7.3	0.29
10	63	F	73	15	Glyburide	2.5, 5.0, 2.5 mg	1200	53	16.4, 6.7	0.26
11	67	M	54	1	Gliclazide	40 mg	1600	49	18.6	0.22
					Gliclazide	20 mg	1400	35	6.8	0.24
12	70	F	55	20	Insulin	14 U	1400	40	12.9	0.25
					Gliclazide	80 mg	1200	18	6.2	0.31
13	74	M	47	14	Diet only		1200	55	12.4, 6.4	0.30
14	82	F	46	8	Glyburide	2.5 mg	1200	60	15.5, 6.0	0.28

\*During treatment.

†Treatment started with initial drug for described days and then, in some patients, changed to the next prescription during treatment period.

‡The former is the value at the beginning, and the latter is the value at the end during treatment of the patient.

of the anion exchanger (AG1 X8). Then the column was washed with 3 ml of water, and all of the eluate was collected and evaporated with a centrifugal evaporator (model 10-E, Tomy Seiko, Tokyo). The dry residue was peracetylated for GCMS analysis. A gas-liquid chromatograph (model GC-14A, Shimadzu, Kyoto) was fitted with a fused silica capillary column (HiCap-CBP1, Shimadzu). Isothermic chromatography was carried out at 177°C. The isothermic elution was followed by column cleaning at 260°C for 5 min. The AG amount in these samples was determined by the method previously described (14). The interassay coefficient of variation (C.V.) was 2.1%, and the C.V. within an assay set was 1.4%.

**Calculation for rate of decrease in plasma AG.** Because we had data from a large sample size, we postulated that if urinary glucose excretion were 0, the plasma AG would increase within the range under its renal threshold. Furthermore, the recovery potency looked similar among the patients, especially under  $\sim 12 \mu\text{g/ml}$  of AG level, and the average increasing rate was  $\sim 0.3 (0.296 \pm 0.018) \mu\text{g} \cdot \text{ml}^{-1} \cdot \text{day}^{-1}$ . Thus, if the plasma AG level was assumed to be on the recovery course, the daily decrease rate of plasma AG was calculated depending on the decrease from the level of 1 day (24 h) before plus  $0.3 \mu\text{g/ml}$  (the expected daily increase), i.e.

daily decrease rate (%)

$$= \frac{\text{decrease}}{\mu\text{g/ml value of former day} + 0.3 \mu\text{g/ml}} \times 100$$

where

$$\text{decrease } (\mu\text{g/ml}) = (\mu\text{g/ml value of former day} + 0.3 \mu\text{g/ml}) - \mu\text{g/ml value at the day}$$

If the plasma AG level was the same as on the former day during the recovery period, the plasma AG was regarded as lost.

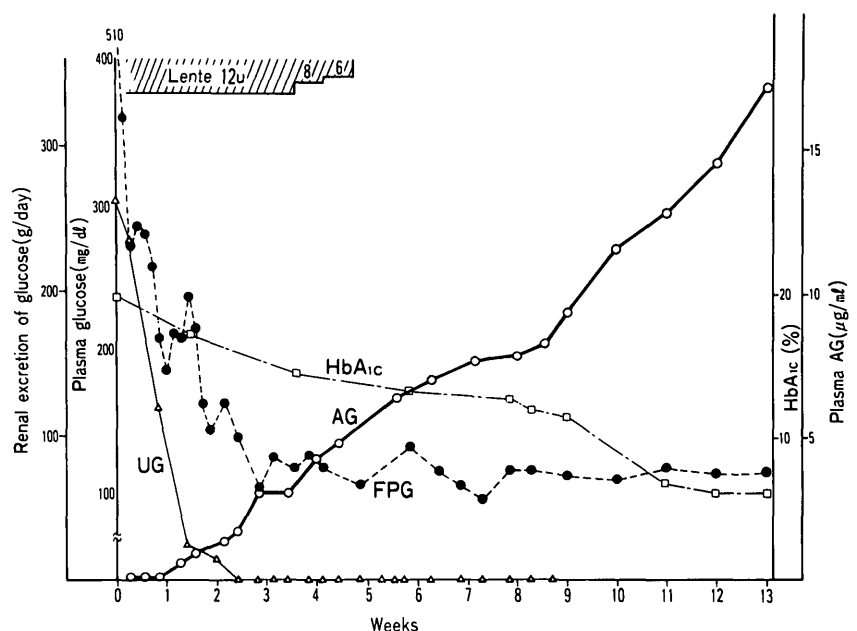
**Statistical analyses.** All data were stored on an NEC PC-9801 computer for analysis. The statistical significance of differences was analyzed by Student's *t* test. Statistical analyses included Pearson, Spearman, and Kendall  $\tau$ - $\beta$  correlation coefficients.

## RESULTS

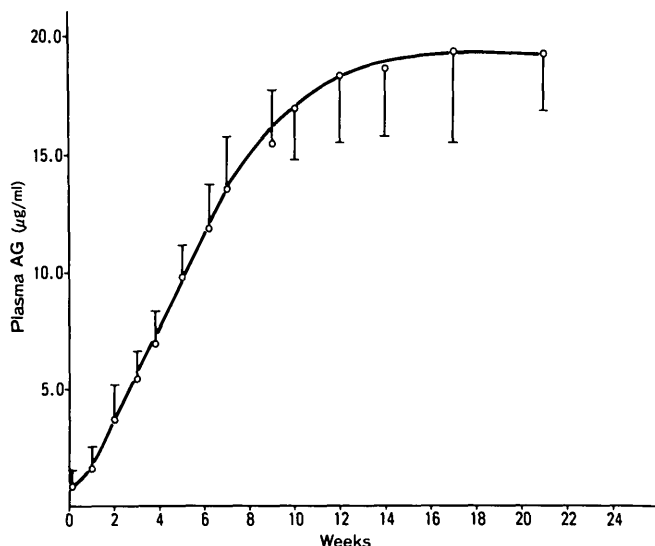
**Time course of recovery of plasma AG concentration after medication for NIDDM.** The results from one typical NIDDM patient after the initiation of treatment confirmed our previous report that recovery of the AG concentration shows some delay after normalization of the FPG and  $\text{HbA}_{1c}$  levels (Fig. 1). Furthermore, we observed that the plasma AG level recovered, with a linear curve, more rapidly and steadily with more strict glycemic control. We proved also that the technique used for measurement of AG could offer reliable and accurate data that might demonstrate a slight change occurring during even 1–2 days.

**AG recovery curve.** The time course of plasma AG recovery in NIDDM patients whose glycemic levels were strictly controlled is shown in Fig. 2. All patients showed normal or near-normal monthly  $\text{HbA}_{1c}$  levels 6–10 wk after starting treatment. Daily urinary glucose, measured at least once per 2 or 3 wk also fell to  $<1 \text{ g/day}$  (NS) 6 wk later. In this group, plasma AG showed constant recovery rate. The rate of increase in plasma AG was markedly similar among recovering NIDDM patients (including 1 with gestational diabetes mellitus) whose glycemic control had remained excellent. This similarity lasted for at least 7–10 wk after initiation of the plasma AG recovery. The rapid increase in the plasma AG level was followed by a slow increase or a (near-) plateau curve, and the plateau points for plasma AG were widely different, as in the case of the plasma AG concentrations in normal subjects. On the other hand, in some patients the plasma AG recovery pattern was accompanied by deterioration of plasma glucose (data not shown).

**Constant rate of increase in plasma AG.** The daily increase rate of plasma AG was constant in and between individuals,



**FIG. 1.** Changes in fasting plasma glucose (FPG),  $\text{HbA}_{1c}$ , urinary daily glucose (UG), and plasma 1,5-anhydro-D-glucitol (AG) concentration in NIDDM patient with poorly controlled glycemia after starting insulin treatment. Clinical details of patient are shown in Table 1 (patient 8).



**FIG. 2. Time course of normalization of plasma 1,5-anhydro-D-glucitol (AG) levels in NIDDM patients with poorly controlled glycemia after strict control of plasma glucose. Clinical details of patients are shown in Table 1. Data are means ± SE (n = 14).**

especially after excellent glycemic control had been attained (Fig. 2; Table 1). If we considered the period in which the glycemic control was excellent, that is, when urinary glucose excretion was negligible, the daily increase rate of plasma AG was  $0.296 \pm 0.018$  (mean ± SD; range 0.23–0.35; n = 82 patients), which appeared constant. Furthermore, the rate does not seem to have been influenced by treatment (diet, drugs, or insulin therapy), sex, age, body weight, duration of diabetes mellitus, or plasma AG level (Table 1).

**Relationship between decrease in plasma AG and glucosuria.** The decrease in plasma AG seemed to show a close correlation with urinary glucose in the longitudinal observation. If urinary glucose was absent or negligible, the increase rate of plasma AG was  $\sim 0.3 \mu\text{g}/\text{day}$ ; however, the rate decreased according to the increase in urinary glucose (data not shown).

Assuming  $0.3 \mu\text{g}/\text{day}$  to be the average upper limit of the increase rate in a day, we found a fine correlation between the degree of the decrease in plasma AG ( $\Delta\text{AG}$ ) and the renal excretion of glucose. With the calculation for the rate of decrease in plasma AG (MATERIALS AND METHODS), we found a high correlation between the percentage of the decrease in AG and urinary glucose ( $r = .853$ ), and furthermore, the correlation was not affected by the level of plasma AG itself (Fig. 3). The calculation enabled us to apply the hypothesis of the close correlation between  $\Delta\text{AG}$  and urinary glucose even in the early phase of recovery from glycemic disarrangement accompanied by a high degree of glucosuria (data not shown).

**A · G index.** According to the calculation for the rate of decrease, the continuous subnormal constant level of plasma AG implies the existence of a constant urinary glucose level. For example,  $\Delta\text{AG}$  (x%) is calculated from the plasma AG concentration A in such patients

$$x(\%) = \frac{(A + 0.3) - A}{A + 0.3} \times 100 = \frac{0.3}{A + 0.3} \times 100$$

If we assume that 70 g of urinary glucose per day would make  $-\Delta\text{AG} = 100\%$  (Fig. 3), the amount (g) of urinary glucose G is

$$70 \times \frac{x}{100} = G \text{ (g)}$$

The equation can be solved with respect to A and G

$$(A + 0.3) \times G = 21$$

Because  $A \gg 0.3$  in the ordinary state, this equation can be simplified

$$A \times G \cong 21$$

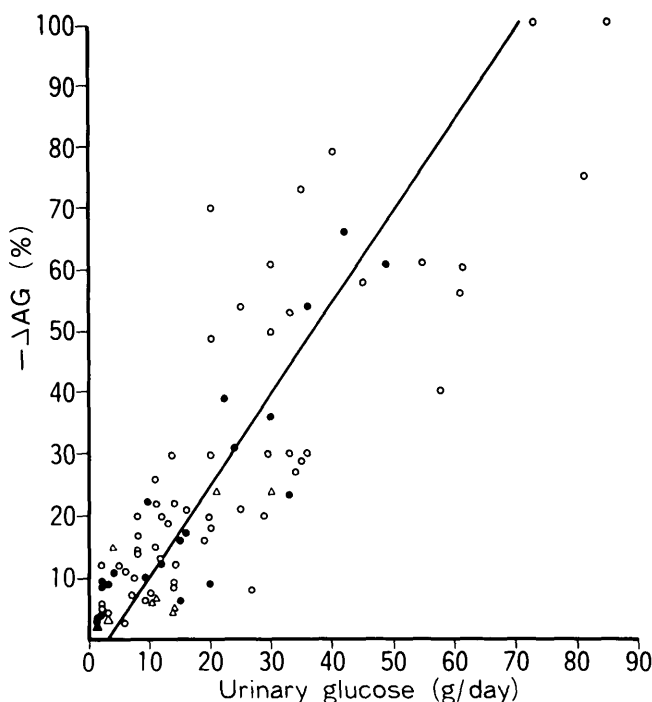
We tested this hypothesis. The data for many patients with stable plasma AG and urinary glucose levels supported this assumption (Fig. 4). The calculated  $A \times G$  was  $16.1 \pm 3.4$  (mean ± SD) in 47 patients. Therefore, from these clinical studies

$$A \times G = 16$$

is a simple equation for the rough estimation of urinary glucose from the plasma AG concentration. We call this the  $A \cdot G$  index.

**Relationship between plasma AG, FPG, HbA<sub>1c</sub>, and urinary glucose in patients with stable glycemic control.**

The plasma AG concentration correlated clearly ( $P < .0001$ ) with FPG ( $r = -.810$ ) and HbA<sub>1c</sub> ( $r = -.856$ ) in the same group shown in Fig. 4 (Fig. 5). By the computer analysis, a



**FIG. 3. Correlation between percentage of decreased degree of plasma 1,5-anhydro-D-glucitol ( $-\Delta\text{AG}$ ) and amount of urinary glucose in 24 h. Each case is grouped by plasma AG value at measurement: O,  $<2.0 \mu\text{g}/\text{ml}$ ; ●, between 2.0 and 5.0  $\mu\text{g}/\text{ml}$ ; Δ, between 5.0 and 10.0  $\mu\text{g}/\text{ml}$ ; ▲,  $\geq 10.0 \mu\text{g}/\text{ml}$ .  $y = 1.48x - 4.92$ ;  $r = .853$ .**

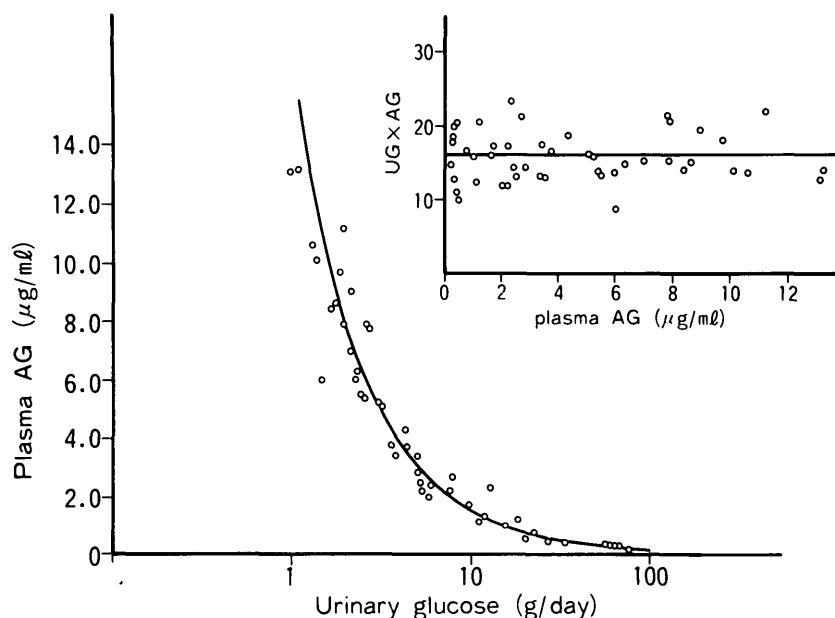


FIG. 4. Correlation between average plasma 1,5-anhydro-D-glucitol (AG) level and amount of urinary glucose in NIDDM patients whose glycemic control and plasma AG levels had been stable during preceding 3 mo. Plasma AG and urinary glucose are shown as averages for 3 samples collected at 4-wk intervals. Curve indicates plasma AG  $\times$  urinary glucose = 16. Inset: urinary glucose value (UG) multiplied by AG value; values are plotted by plasma AG level. Solid line indicates average multiplied value.

noticeable enhancement in correlation resulted. The Spearman correlation coefficient for AG and FPG was  $-.902$  ( $P < .0001$ ) and for  $HbA_{1c}$  was  $-.910$  ( $P < .0001$ ). For urinary glucose, the Spearman correlation coefficient was  $-.976$  ( $P < .0001$ ), and the Kendall  $\tau$ - $\beta$  was  $-.890$  ( $P < .0001$ ). The range 1–10  $\mu\text{g/ml}$  for AG value corresponds to the range 100–230 mg/dl for FPG and 6–11% for  $HbA_{1c}$  (Fig. 5).

#### DISCUSSION

Our results indicate that the plasma AG level sensitively reflects the urinary excretion of glucose in NIDDM patients. Although neither the physiological significance nor the pathway for metabolism of AG has been clarified, the turnover rate of plasma AG is known to be low (1,7), and the rate of de novo AG synthesis is expected to be low. On the other hand, because AG has a similar structure to glucose, it was conjectured that some cells or tissues cannot distinguish between these two compounds. In fact, we recently ob-

served that AG was transported in renal tubules and that it might be competitively inhibited by glucose in rats, and we confirmed that the reduction of plasma AG in diabetes mellitus is mainly due to accelerated urinary excretion, which is coincident with the excretion of glucose (unpublished observations). Our data confirmed that a similar mechanism may be present in humans. The origin of AG in the body was unknown; until this study, however, it had been suggested that AG partly originates from orally ingested food (unpublished observations). However, because there is a large pool of AG in the body (14), the balanced state of the AG level in tissues and intravessels is probably little affected by AG production or oral intake.

In this study, we showed that the increase rate of plasma AG is usually  $\leq 0.3 \mu\text{g/day}$ , which is surely much less than the normal plasma AG concentration. Furthermore, this large pool and metabolic inertness of AG will explain the small but constant increase in plasma AG after treatment for glycemic disarrangement. The increase in AG is followed thereafter

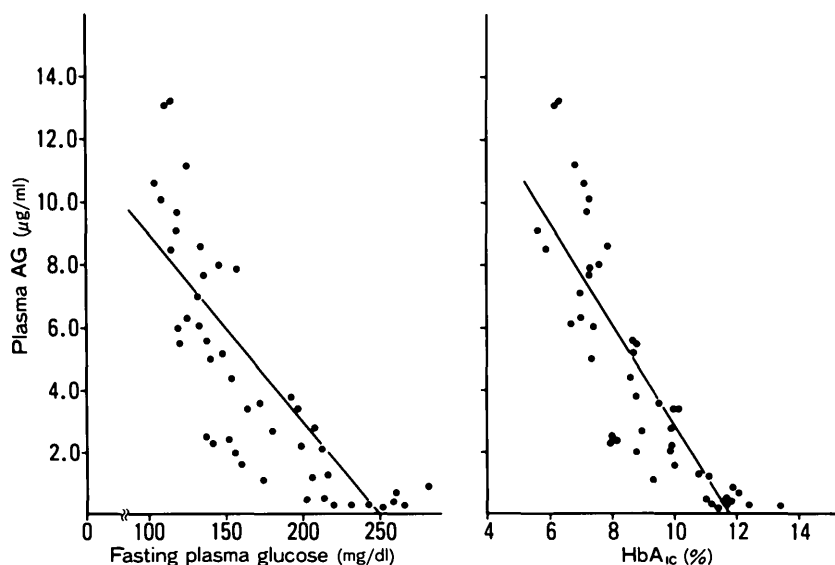


FIG. 5. Correlation between average plasma 1,5-anhydro-D-glucitol (AG) level and average fasting plasma glucose (FPG) and  $HbA_{1c}$  in same group shown in Fig. 4. Plasma AG, FPG, and  $HbA_{1c}$  are shown as averages for 3 samples collected at 4-wk intervals. FPG:  $n = 47$ ,  $y = 0.06x + 15.01$ ,  $r = .810$ ,  $P < .0001$ .  $HbA_{1c}$ :  $n = 47$ ,  $y = 1.61x + 18.97$ ,  $r = .856$ ,  $P < .0001$ .

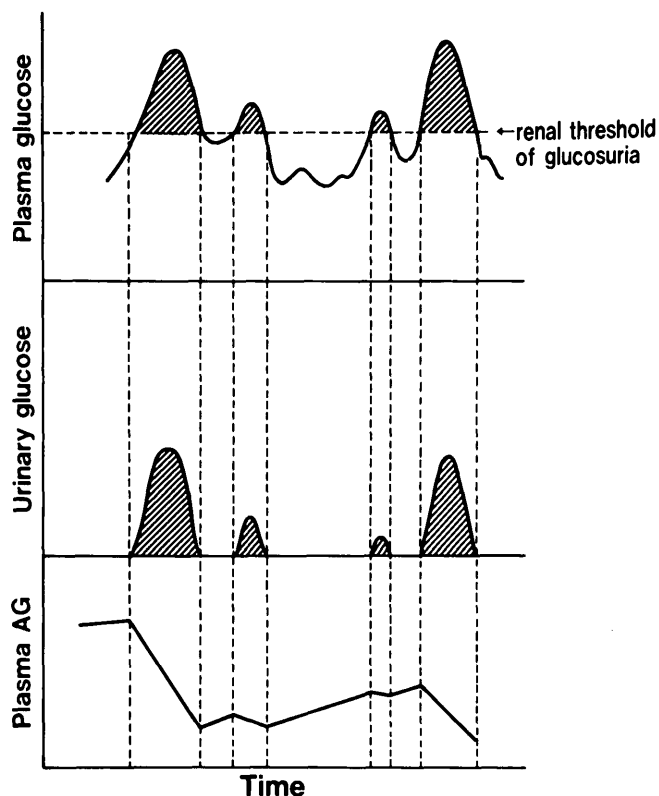


FIG. 6. Schematic representation of mechanism determining plasma 1,5-anhydro-D-glucitol (AG) level.

by a plateau state, which may be determined by the renal threshold of AG (7). All of the observations indicating that the change in plasma AG is mainly influenced by glucosuria are compatible with the previous results showing that the AG level was specifically reduced in diabetes mellitus and little influenced by various factors, such as lipid metabolism, liver dysfunction, renal dysfunction (not severe cases), various hormones, body mass, sex, or age (5). Taken together, these observations indicate that the mechanism underlying that plasma AG change is as shown in Fig. 6. Moreover, plasma AG is markedly influenced by urinary excretion of glucose, and the reduction is so sensitive and rapid that the plasma AG can be used as an early marker for deterioration of glycemic control.

We adopted a special method of calculating the decrease in the level of plasma AG. If we did not use this method, the correlation between  $\Delta AG$  and urinary glucose would not have been a proper calculation, because several patients showed an increase in plasma AG despite some degree of urinary glucose excretion. This method is useful particularly for explaining the AG increase in an early phase after starting treatment, when a significant extent of glucosuria exists. Furthermore, the application of this method does not interfere with interpretation of the  $A \cdot G$  index. If the plasma AG level is maintained at  $2.0 \mu\text{g/ml}$  for some time,  $\Delta AG$  is regarded to be  $(0.3/2.3) \times 100 = 13\%$ , which means  $\sim 6\text{--}14 \text{ g}$  of daily urinary glucose according to the distribution shown in Fig. 3. On the other hand, from the  $A \cdot G$  index, an AG level of  $2.0 \mu\text{g/ml}$  means that there is  $\sim 8 \text{ g/day}$  of continuous urinary glucose. Similarly, if the plasma AG level is  $8.0$

$\mu\text{g/ml}$ , urinary glucose should be  $\sim 1.5\text{--}5 \text{ g/day}$ , and  $2 \text{ g}$  is expected from the  $A \cdot G$  index (Fig. 3).

Finally, several points remain to be resolved by further studies. First, there is some ambiguity regarding the relationship between AG and urinary glucose in a period of low plasma AG level, such as when the AG level is  $< 0.1 \mu\text{g/ml}$ . Second, we excluded patients with severe renal failure, renal glucosuria, and drug (especially steroid)-induced glucosuria. Regarding these patients, we have the impression that there is a relationship between the plasma AG level and urinary glucose itself even in severe uremia or renal glucosuria; however, the rate of decrease in plasma AG per certain amount of glucose excretion, especially in patients with renal or steroid-induced glucosuria, may be different from that in typical NIDDM patients. However, these diseases are not common, so we have not been able to confirm this impression. Finally, we excluded insulin-dependent diabetes mellitus (IDDM) in this study. We have shown that the AG concentration is significantly lower in IDDM than in NIDDM (8). The assessment of the data for IDDM patients is in progress, and the results will be described elsewhere.

The results of this study demonstrated that the reduction in the plasma AG concentration closely reflects the extent of glucosuria in NIDDM patients and suggested that urinary glucose can be roughly determined under continuous similar glycemic control with the  $A \cdot G$  index. The plasma AG value also showed marked correlations with all the other diabetic indicators examined and, accordingly, is expected to serve as an additional indicator of the glycemic control of diabetes. Furthermore, some properties give AG measurement the advantage over traditional tests of serving as a marker of glycemic control. First, AG has an analytical character as described in this study. Second, the AG value is not as relative a value as  $\text{HbA}_{1c}$ . Finally, the AG value varies between wide limits corresponding to the range  $6\text{--}11\%$  for  $\text{HbA}_{1c}$ . This variation means that AG would be influenced little by variation of the measurement compared with  $\text{HbA}_{1c}$ . For many NIDDM patients with such fair glycemic control, the correct and fine assessment of their glycemic conditions would be a crucial problem. Thus, the measurement of plasma AG may be of clinical significance.

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