Reduction and Recovery of Plasma 1,5-Anhydro-D-Glucitol Level in Diabetes Mellitus

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SUMMARY

The plasma concentration of 1,5-anhydro-D-glucitol (AG) was measured in 135 newly diagnosed patients who were referred for oral glucose tolerance tests. AG concentrations in the nondiabetic patients indicated that the mean value of normal AG concentration was 21.8 μg/ml (SD = 5.9 μg/ml, range 9.6–38.8 μg/ml). This distribution of AG concentration was significantly different from that in patients with impaired glucose tolerance (IGT) (13.3 ± 5.4 μg/ml) and definitely different from that in diabetic patients (2.1 ± 1.8 μg/ml). In a standard glucagon test, it was suggested that the decrease of plasma AG was affected not only by glycemic control of the patients but also by pancreatic cell secretory activity. The reduction of AG concentration was more marked in IDDM patients than in NIDDM patients. In longitudinal studies, AG concentration was shown to be sensitive to glycemic control. However, its recovery showed a tendency toward much delay after the improvement of fasting blood glucose or HbA_1 concentrations. On the other hand, AG concentration showed negligible diurnal change and no immediate change as a result of diet, oral glucose load, or acute shift of the insulin level in both normal and diabetic subjects. *Diabetes* 36: 709–15, 1987

The compound 1,5-anhydro-D-glucitol (AG) is a six-carbon monosaccharide with a pyranoid ring structure resembling glucose. This compound is one of the main polyols in human cerebrospinal fluid (1) and serum (2) but is not excreted into urine under normal conditions (3). A low AG concentration in blood has been shown to be associated with diabetes mellitus (1,2,4,5). Pitkänen (6) observed low AG concentrations in newly diagnosed diabetics and in diabetics with a long history of the disease, regardless of diabetic nephropathy. Recently, we confirmed that the plasma AG level was reduced to zero in streptozocin-induced diabetic rats and that the AG level was partially restored by insulin therapy (7). Although a low AG concentration in plasma has been proposed to be an indication of metabolic disarrangement in diabetes mellitus (8), no detailed study has been reported on the relationships between AG concentration and other traditional markers or glycemic control in diabetic patients. We studied the effect of long-term glycemic control on AG concentration in patients with established diabetes and the effect of food or oral glucose load on diurnal change of AG concentration. We also examined the difference in AG concentration between IDDM and NIDDM patients.

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SUBJECTS AND METHODS

Subjects. The plasma AG concentration was examined in 135 newly diagnosed subjects (72 men, 63 nonpregnant women), 18–81 (mean 49) yr old, who were referred for glucose tolerance tests. These subjects comprised 45 without diabetes, 22 with impaired glucose tolerance (IGT), and 68 with diabetes. Classification was made according to criteria described below. The three groups were not statistically different in mean age and gender distribution. The nondiabetic group mainly comprised people who came to the hospital for thorough physical examinations. No member of this group had a personal or family history of diabetes, and random blood glucose concentrations were normal. The correlation of AG concentration with HbA_1 levels or C-peptide concentrations after glucagon injection was examined in 127 subjects selected from the patients whose glycemic control was stable for at least the last 4 mo; i.e., the variance of HbA_1 levels was within ±10% during that period. These subjects comprised 30 nonobese patients with IDDM and 97 patients with NIDDM (6 were being treated with insulin plus

TABLE 1
Characteristics of IDDM and NIDDM patients

	IDDM (n = 30; 18M, 12F)	NIDDM (n = 97; 59M, 38F)
Age (yr)	30 ± 3	55 ± 8*
Duration of diabetes (yr)	10.4 ± 2.0	4.8 ± 1.6*
Daily insulin dose (U/day)	35 ± 4	0
Plasma free–C-peptide response to intravenous glucagon (6-min value; ng/ml)	0.42 ± 0.36 (range 0–1.10)	3.40 ± 1.73* (range 1.09–8.5)

Values are means ± SE.
*$P < .01$ vs. patients with IDDM.

sulfonylureas, 50 with diet and sulfonylureas, and 41 with diet alone). Their clinical characteristics are given in Table 1. Some of these patients had various complications arising from diabetes mellitus, but patients with other diseases were excluded.

Longitudinal studies were carried out on 10 male and 4 female NIDDM patients, 30–78 (mean 52) yr old, who had shown remarkable improvement or worsening of glycemic control during the last 2–4 mo. Clinical details of these patients are summarized in Table 2. To study the diurnal alteration of AG, glucose, and HbA_1 concentrations, venous blood samples were taken from eight subjects, including six diabetic patients, one IGT patient, and one normal subject, at 0800, 1200, 1400, 1600, 2000 h, and midnight.

Diagnosis. A standard 2-h oral 75-g glucose tolerance test was performed after overnight fasting. Based on criteria established by the National Diabetes Data Group (9) and the World Health Organization Expert Committee (10), subjects were classified into three groups: diabetics, IGT subjects, and nondiabetics. IDDM was defined by susceptibility to ketoacidosis, with a permanent need for insulin that developed within 6 mo after initial diagnosis. NIDDM subjects had neither ketoacidosis within the 1st yr after diagnosis nor a permanent need for insulin. The discrimination of IDDM and NIDDM was confirmed by the analysis of free–C-peptide response to glucagon as described before (11).

Assays. Glucose concentration was determined with a Fuji DRI-CHEM 2000 analyzer (Fuji, Tokyo) (12). Glycosylated hemoglobin was assayed with a column system provided by Bio-Rad (Richmond, CA). All column assays were carried out in a water bath maintained at 23°C, and equality control was assessed on the basis of variability of the data for fresh normal samples. Insulin radioimmunoreactivity (IRI) was determined by the method of Yalow et al. (13). Standard glucagon tests were performed by the method of Poulsen et al. (11), where serum free–C-peptide reactivity (CPR) was determined in the blood obtained 6 min after a 1-mg i.v. glucagon load, according to the method described by Hsieh and Akanuma (14).

Plasma AG concentration was determined by gas-liquid chromatography (GLC). Plasma (0.1 ml) was first added to 5 μg of xylitol, which served as an internal standard, and was then mixed with 1 ml of methanol. The methanolic extract obtained after centrifugation was dried in a vacuum and was further purified by partition between water and chloroform. The aqueous layer was dried in a vacuum, and the resulting residue was acetylated with 0.3 ml of acetic anhydride/pyridine (1:2 vol/vol). After standing overnight at room tem-

TABLE 2
Clinical details of patients in longitudinal study

Case	Age (yr)	Sex	Duration of diabetes (yr)	Complications	Treatment
1	55	F	9	Neuropathy	5 mg glibenclamide
2	49	M	25	Proliferative retinopathy Neuropathy Nephropathy	2.5 mg glibenclamide
3	65	M	9	Background retinopathy	2.5 mg glibenclamide
4	57	M	1	Neuropathy Background retinopathy	2.5 mg glibenclamide
5	55	M	0		20 U insulin
6	50	M	1		2.5 mg glibenclamide
7	78	M	6		2.5 mg glibenclamide 100 mg buformin
8	45	M	3		47 U insulin
9	52	M	0	Neuropathy	1.25 mg glibenclamide
10	34	M	0		1.25 mg glibenclamide
11	60	M	9		7.5 mg glibenclamide 100 mg buformin
12	72	F	1	Background retinopathy	Diet
13	30	F	0		22 U insulin
14	31	F	0		40 U insulin

Patients 1–6 as seen in Fig. 3, *top*; patients 7–13 as seen in Fig. 3, *bottom*; patient 14 is described in Fig. 4.

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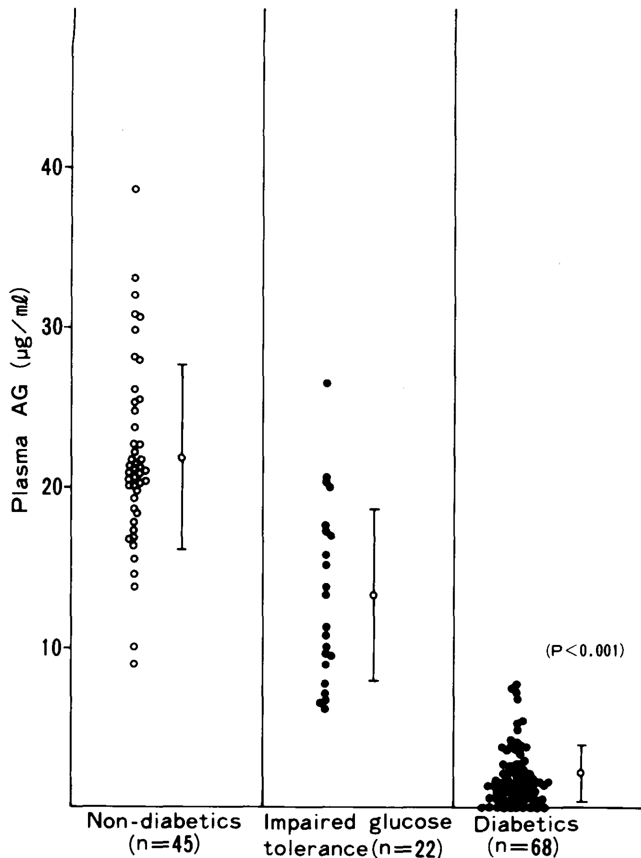

FIG. 1. Scattergram of 1,5-anhydro-D-glucitol (AG) concentrations in plasma from normal subjects, impaired glucose tolerance patients, and diabetic patients. Criteria of World Health Organization were used to distinguish subjects. *Bars*, means ± SD; $P < .001$ between 1st and 3rd groups.

perature, the reaction mixture was added to 1 ml of water to hydrolyze the anhydride, and the acetylated materials were extracted with ether, which was then removed by evaporation. The acetylated preparation was subjected to isothermic GLC (203°C) on 3% SP 2340 (Superco, Bellefonte, PA) on Chromosorb W packed in a 2-m glass column. The gas chromatograph used was a model GC-4CMPF (Shimadzu Seisakusho, Kyoto, Japan). The precision of the method was checked by repeating the measurement of a standard sample made of a known amount of authentic AG and a diabetic plasma originally containing no detectable AG amount (50 μg/ml plasma). One or two tubes of the standard sample were always included in each set of AG assays. The C.V. calculated from the data for the standard sample was 3.9% (mean 53.7 μg/ml plasma, $n = 85$). The C.V. within a single assay set was much smaller: 1.4% (mean 53.9 μg/ml, $n = 13$). Note that AG recovery in the standard sample apparently exceeded 100%. We separately demonstrated that this was due to the presence in the plasma samples of unidentified contaminant(s) that interfered more with the perfect acetylation of xylitol, the internal standard, than with that of AG (unpublished observation). However, the observed values were used without correction for this determinate error because this error was not much larger than the variation of the measurements by our method.

Statistics. Probability of rejection of null hypothesis (P) was calculated from Student's t test.

RESULTS

Plasma AG concentration and diabetes mellitus. The distribution of AG concentration was markedly altered among the three groups of patients referred for oral glucose tolerance tests (Fig. 1). The AG concentration in the nondiabetic patients (21.8 ± 5.9 μg/ml, mean ± SD) was significantly ($P < .01$) different from that in the IGT patients (13.3 ± 5.4 μg/ml) and definitely different ($P < .001$) from that in the diabetic patients (2.1 ± 1.8 μg/ml). Assuming 14 ± 1.5 μg is the lower limit of the normal range, all 68 diabetic patients were distinguished from normal patients, and 13 of 22 IGT patients were different on the basis of plasma AG concentration.

To explore whether the reduction of AG reflects differences in the type of diabetes (IDDM or NIDDM), we also performed a standard glucagon test in 127 patients selected on the basis of glycemic control stability and compared the free-C-peptide values obtained 6 min after a 1-mg i.v. glucagon load with the AG values determined in these patients (Fig. 2). The states of glycemic control are divided into three

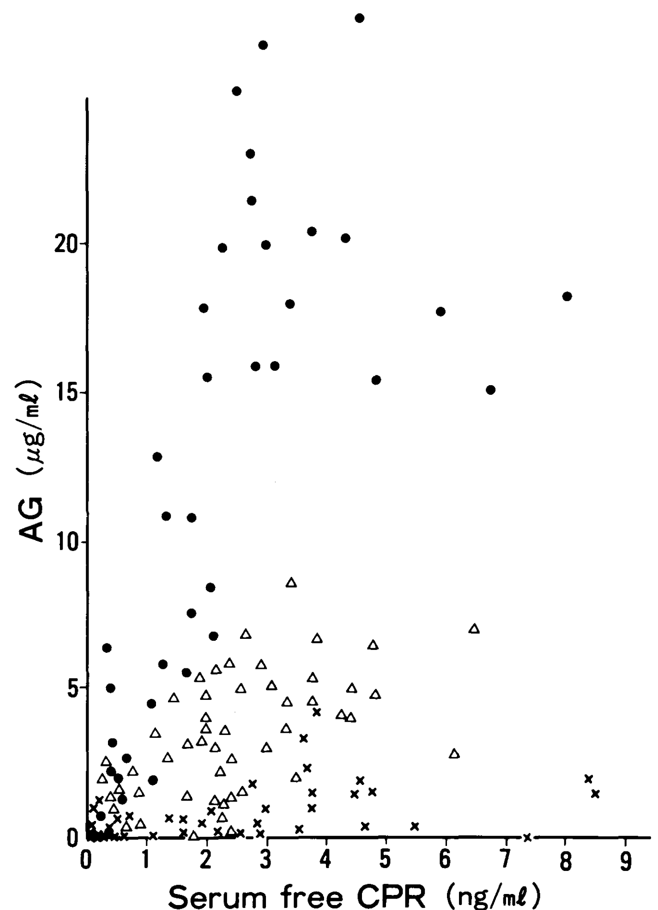

FIG. 2. Scattergram of 1,5-anhydro-D-glucitol (AG) concentration vs. 6-min C-peptide values in 127 subjects whose glycemic control had been stable. Subjects are grouped by average fasting plasma glucose during 3 mo before AG measurement: ●, <140 mg/ml; △, between 140 and 200 mg/ml; X, ≥200 mg/ml.

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TABLE 3
Comparisons of plasma 1,5-anhydro-D-glucitol (AG) concentrations between IDDM and NIDDM patients under similar glycemic control

HbA_1 (mean %)	Plasma AG (μg/ml)			
	IDDM	n	NIDDM	n
<8.0			16.5 ± 2.9	18
8.1~9.0	3.1 ± 1.6	4	8.0 ± 3.3*	13
9.1~10.0	2.0 ± 1.1	8	4.2 ± 1.7*	27
10.1~12.0	0.6 ± 0.9	11	1.7 ± 0.9*	27
>12.1	0.2 ± 0.2	7	1.0 ± 0.7*	12

Patients were selected as described in text. Values are means ± SE.
*$P < .01$ vs. IDDM patients.

groups based on the mean value of fasting plasma glucose concentrations during the foregoing 3 mo (see various symbols in Fig. 2). Generally, the better glycemic control a patient attained, the higher the plasma AG value. However, the patients with low free–C-peptide responses were prone to lower AG values, even under good glycemic control. Especially when the C-peptide value of a patient was <1.0 ng/ml, the AG value seemed to rarely exceed 7 μg/ml, even if the average fasting plasma glucose remained <140 mg/ml. This result may be interpreted in terms of a very low plasma AG level in IDDM patients regardless of glycemic control. This was further confirmed by a direct comparison of AG values between IDDM and NIDDM patients under similar glycemic control (Table 3). The comparison of patients with similar HbA_1 values apparently shows that AG concentration is significantly lower in IDDM than NIDDM.

Longitudinal study. Figure 3 compares alterations of plasma AG, fasting blood glucose, and HbA_1 concentrations in 13 NIDDM patients who showed either remarkable improvement in glycemic state within 4 mo or noticeable recession within 2 mo. All the patients who showed marked reductions of fasting plasma glucose and HbA_1 also showed pronounced increases in AG concentration (Fig. 3, *top*). The changes in the three levels averaged 52 and 37% reductions and an eightfold increase (from 1.5 to 11.8 μg/ml), respectively. Conversely, increases in the levels of fasting blood glucose and HbA_1 were accompanied by decreases in AG concentrations. These increases and decreases averaged 23, 14, and 57% (from 7.7 to 3.3 μg/ml), respectively. Thus, AG concentration showed a marked response to the glycemic state in various types of diabetes (cf. Table 2).

The recovery of AG concentration showed a distinct delay after normalization of fasting blood glucose and HbA_1 levels. Such an observation is exemplified in Fig. 4, which shows changes in the three concentrations in a single patient for 37 wk after the start of insulin treatment. With continual glycemic control by insulin, the concentration showed steady restoration from nadir along with the recoveries of fasting blood glucose and HbA_1 concentrations. Although both of the established markers were normalized by the 23rd wk, AG concentration was still far from the normal range. The patient lost glycemic control at the 27th wk due to misuse of insulin. The resulting recession of the glycemic state was well reflected in the two established glycemic markers. Concomitantly, AG concentration decreased sharply, almost down to the initial level. The shifts of AG and fasting blood glucose concentrations started immediately after the 27th wk, whereas the increase in HbA_1 concentration showed an ~3-wk delay.

Diurnal change of AG concentration. AG concentration seemed to undergo negligible acute changes in response to the plasma concentrations of either insulin or glucose. No marked change of AG concentration was observed after an oral load of 75 g glucose (Fig. 5). As illustrated, the oral glucose load generally caused a rapid increase in blood glucose, which is normally followed by an immediate increase in blood insulin. Accordingly, this stability of AG concentration indicated that neither glucose nor insulin in the blood had an acute effect on AG concentration. Because the data in Fig. 5 include all three groups of patients, the short-range stability of AG concentration appears to be independent of the type of diabetes. AG concentration underwent little diurnal change. No significant change was observed within a day in any of the eight subjects, including nondiabetic, IGT, IDDM, and NIDDM patients (Table 4).

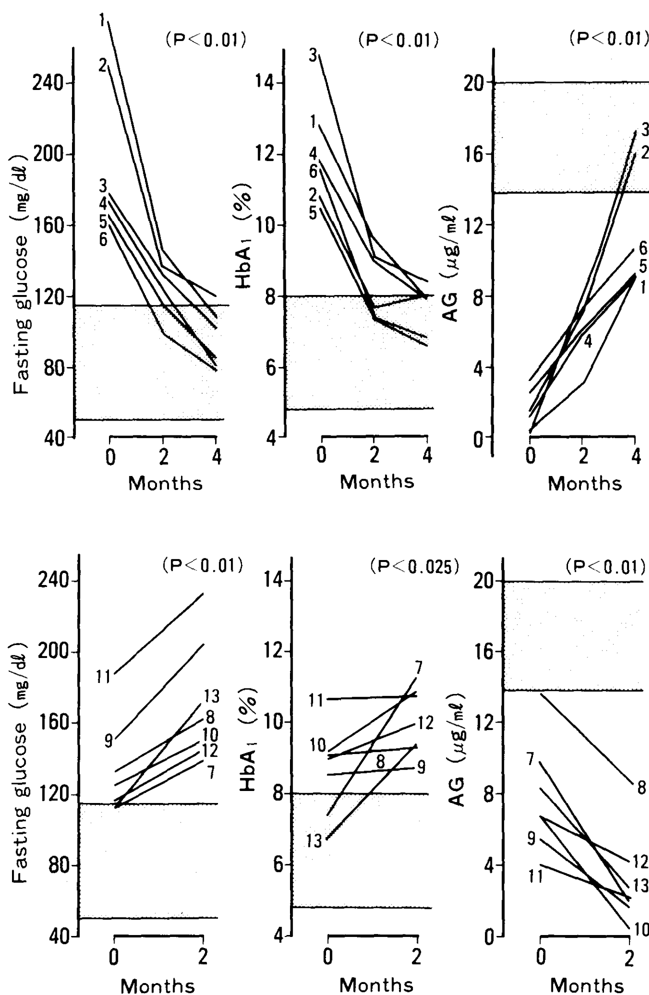

FIG. 3. Changes in fasting blood glucose, HbA_1, and plasma 1,5-anhydro-D-glucitol (AG) concentrations over 2–4 mo in 13 patients (clinical details are shown in Table 2). Patients identified by number given in table and figure. *Top*: 6 patients who showed improved diabetic control. *Bottom*: 7 patients showing deterioration. *Horizontal lines*, boundary of respective normal range.

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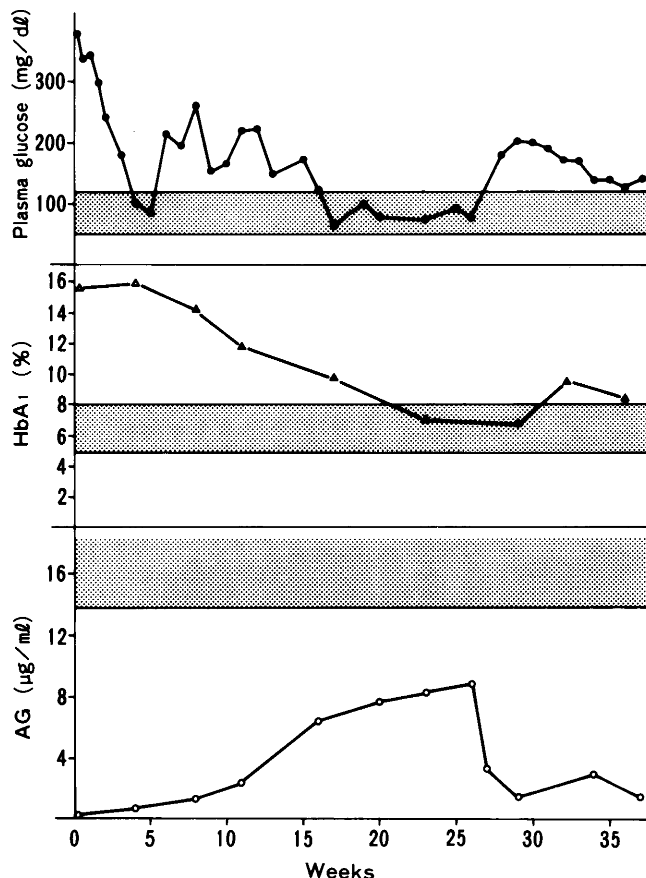

FIG. 4. Changes in fasting blood glucose, HbA_1, and plasma 1,5-anhydro-D-glucitol (AG) concentrations in patient with newly diagnosed diabetes after starting insulin treatment. *Horizontal lines*, boundaries of normal ranges. Clinical details of patient are shown in Table 2 (case 14).

DISCUSSION

All the data presented herein support the previously reported concept that diabetes is accompanied by a reduction in plasma AG concentration. Our results indicate that the normal range of AG concentration is 9.6–38.8 μg/ml (mean ± SD, 21.8 ± 5.9 μg/ml). However, this value is considerably different from previously reported normal ranges. Values appearing in the literature are 1.5–24 μg/ml (in our unit; 6), 13.3 ± 0.41 (SD) μg/ml (we wonder if SD is a misprint of C.V.) (15), and 25 ± 9 μg/ml (16). This disagreement may be partly due to the immaturity of the AG assay method for plasma, which contains many monosaccharides and polyols. Our previous AG assay method was based on liquid chromatography (5), which later proved to be unsuitable for AG determination because the flow fluorometry that was employed in the assay system involved periodic acid oxidation to which AG was highly resistant, and the imperfect AG oxidation resulted in considerable degradation in terms of precision and accuracy, as well as sensitivity, of AG determination (17). The use of a polar liquid phase, SP 2340, in GLC made its application in determining plasma AG practical. Although GLC with capillary columns has been successfully applied in determining plasma AG (6,18), we avoided using it because the capillary columns were expected to be less durable when used for repeated injections of the fairly crude preparations in clinical AG measurement. The precision (C.V. ~4%) and accuracy (relative error <8%) indicated by the 85 measurements of the standard sample seem satisfactory because plasma AG levels show such a drastic difference between normal subjects and diabetics. Although the discrepancy in normal AG values should be studied further, it is still plausible that the plasma AG level shows considerable variation among races; our data showed good agreement with that reported by another Japanese research group (16).

Whereas plasma AG concentration showed a marked correlation with glycemic control, it also showed dependency on pancreatic β-cell secretory activity (Fig. 2). This indication is further supported by the data presented in Table 3. A marked difference in AG concentration is seen between IDDM and NIDDM patients whose glycemic controls were indicated to be similar by HbA_1 levels. Although glycemic control may be somewhat less stable in IDDM than in NIDDM patients in general, the possibility does not seem significant, because we selected the patients showing stable glycemic control and observed among them no evidence indicating that the glycemic states of the IDDM patients had been less stable than those of the NIDDM patients before AG measurement. Pitkänen has reported that very low (<10 μM) AG levels were found in IDDM (6), and intensive treatment with insulin or even continuous subcutaneous insulin infusion therapy for several months had little effect on serum AG concentration (6,15). Based on this observation, he suggested that the AG absence with IDDM was independent of the state of glucose metabolism. His suggestion seems to be supported by our study: any patient classified with IDDM in our study showed very low AG concentration regardless of glycemic state. Further studies, however, are necessary to ascertain why AG concentrations are low and less responsive to insulin therapy in IDDM patients.

Plasma AG concentration underwent little diurnal change and was negligibly affected by diet or short-term high insulin or glucose levels. We have also separately demonstrated in humans and rats that the AG value is independent of feeding and starvation (unpublished observations). These observations indicate that the AG value is stable within a day. This stability may be interpreted in terms of the very long half-life (~3 days) reported earlier (19). We recently confirmed the metabolic stability of [^{14}C]AG in blood. After dilution in the circulation, the level of the exogenous AG was highly persistent and underwent little metabolic conversion (7). On the other hand, we also observed that the AG value responded well to long-term glycemic control in NIDDM patients. The results of the 2- or 4-mo observations presented in Fig. 3 clearly show antiparallel relationships of the AG value with fasting glucose and HbA_1 concentrations. A similar antiparallelism was also demonstrated in the longitudinal study shown in Fig. 4 and in the comparison of the plasma AG and HbA_1 concentrations in IDDM and NIDDM patients shown in Table 3. Our recent study also demonstrated that the plasma AG level in rats was reduced to zero with streptozocin administration and that it was partially restored by prolonged insulin treatment (7). These observations indicate that the AG level is a stable indicator that sensitively responds to the duration of glycemic control, especially in NIDDM patients.

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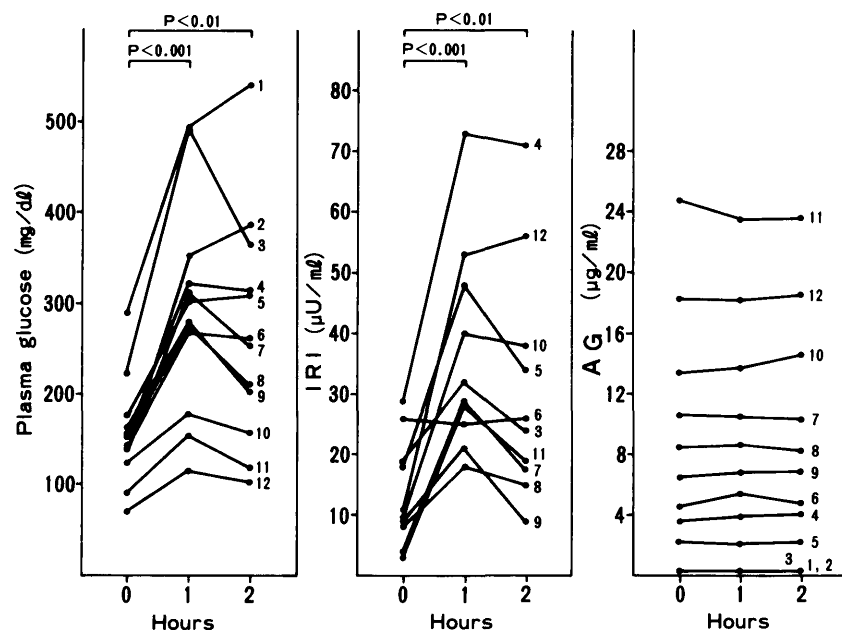

FIG. 5. Changes in blood glucose, immunoreactive insulin (IRI), and plasma 1,5-anhydro-D-glucitol (AG) levels during 75-g oral glucose tolerance tests in 12 subjects. Number shown in each *panel* identifies subject. Nos. 1 and 2 are IDDM patients whose plasma levels of free-C-peptide reactivity was <0.3 ng/ml during glucose tolerance tests. Nos. 3–9 are NIDDM patients (nos. 6–9 were under drug treatment). No. 10 is IGT patient, and nos. 11 and 12 are normal subjects.

Glycosylated hemoglobin is another stable indicator of long-term glycemic control. We show that plasma AG responds to the degree of glycemic control much faster than does HbA_1. As shown in Fig. 4, with misuse of insulin, AG and fasting glucose levels showed immediate antiparallel large shifts, whereas the change in glycosylation showed a delay of several weeks. AG concentration showed a rapid and sensitive fall accompanying the loss of glycemic control. On the other hand, the recovery of AG concentration showed a distinct delay and seemed difficult to normalize compared with those of fasting blood glucose and HbA_1 levels. It has been believed that HbA_1 reflects a long history of glucose concentration in plasma, whereas the AG value is shown to depend not only on the duration of glycemic control itself but also, at least partially, on pancreatic β-cell secretory activity. It is of great interest, therefore, to obtain a concrete conclusion about whether the metabolism of AG in IDDM patients is different from that in NIDDM patients. In any case, very strict glycemic control seemed to be needed to normalize AG concentration even in NIDDM patients.

The AG value certainly reflects the severity of diabetes. However, no mechanism for the shift of AG concentration in diabetes has been reported. The origin and fate of plasma AG is totally unknown. It has been reported that AG is efficiently reabsorbed by the renal tubules (19) and is not excreted into urine (3). Therefore, abnormal AG excretion due to renal complication may explain diabetic AG reduction. This possibility, however, was not supported in our study: most of the diabetic patients had few or no renal complications. Moreover, our unpublished observations indicate that the reduction of plasma AG concentration does not seem to be specifically associated with renal failure. As noted above, AG normally undergoes a very slow turnover in blood (i.e., very slow rates of AG release into and AG depletion from blood). In rats, however, the plasma AG level showed biphasic reduction, and the first phase corresponded to a rapid uptake of circulating radioactive AG into various tissues (7). A rapid AG reduction was also induced with streptozocin (7). These observations suggest that diabetes is associated with the loss of selectivity in AG transport. However, it is still necessary to elucidate the exact AG reduction mechanism essential to an effective application of plasma AG measurement in the diagnosis and therapy of diabetes mellitus.

In summary, our study has demonstrated that the level of plasma AG sensitively changes depending on the derangement of glycemic control and shows slow but steady recovery in the NIDDM patients whose glycemic state is improved. The reduction of AG concentration was more marked in

TABLE 4
Diurnal changes of plasma glucose (PG) and 1,5-anhydro-D-glucitol (AG) levels in 3 IDDM, 3 NIDDM, 1 IGT, and 1 normal subject

	Time					
	Breakfast 0800 ↓	Lunch 1200 ↓	1400	Dinner 1600 ↓	2000	2400
IDDM patients						
PG (mg/dl)	158	246	330	282	416	219
AG (μg/ml)	0	0	0	0	0	0
PG	148	210	236	211	316	124
AG	0.7	0.6	0.5	0.6	0.5	0.7
PG	130	187	248	193	327	136
AG	2.1	2.5	2.3	2.3	2.3	2.4
NIDDM patients						
PG	128	268	130	99	152	157
AG	5.2	5.6	5.1	5.2	5.3	5.4
PG	135	159	163	175	215	175
AG	7.0	6.8	7.0	6.8	7.1	6.9
PG	123	147	175	135	150	140
AG	10.4	9.6	10.6	9.9	10.2	10.4
IGT patient						
PG	105	110	165	97	164	103
AG	14.4	14.2	14.4	13.9	14.1	13.9
Normal subject						
PG	78	112	126	92	136	88
AG	20.2	20.6	20.0	22.1	19.6	20.4

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IDDM patients than in NIDDM patients. On the other hand, it shows diurnal stability and is little affected by acute glycemic changes due to either food or glucose load in both normal and diabetic subjects.

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